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Volume 41 OCTOBER 1961 No. 4

CANADIAN JOURNAL OF PLANT SCIENCE

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Published by the
AGRICULTURAL INSTITUTE OF CANADA
OTTAWA 4, CANADA

CANADIAN JOURNAL OF PLANT SCIENCE

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Authorized as second-class mail, Post Office Department, Ottawa

CANADIAN JOURNAL
OF PLANT SCIENCE

VOLUME 41 OCTOBER 1961 No. 4

RESPONSE OF CERTAIN FORAGE SPECIES ON DIKELAND AND UPLAND TO VARIOUS CLIPPING INTENSITIES[1]

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[Received for publication February 1, 1961]

ABSTRACT

A mixture of timothy, orchard grass, red clover, alfalfa and Ladino clover was seeded on areas of upland and dikeland soil at Nappan, N.S. Over a 3-year period plots were cut at 3-, 4- and 8-week intervals to stubble heights of 1½ and 3 inches. Comparisons were made of forage yields and species contribution under the various cutting intensities on the two soils. Forage yield was 20 per cent greater on dikeland and seasonal distribution of forage was better on dikeland than the upland area with which it was compared. Similar responses between treatments were obtained on both dikeland and upland. Timothy contribution fluctuated widely between high spring values and low fall values while orchard grass regularly contributed about 40 per cent of the forage material. Alfalfa contributed reasonably well initially, but failed to persist under more intense clipping, particularly on dikeland. Ladino clover increased in relative value during the 3 years, performing better on upland under more intense clipping.

INTRODUCTION

The Maritime Provinces are primarily a grass-producing area. Most of the grassland is upland but approximately 76,000 acres is dikeland. Precise information regarding the influence of various management practices on forage species is not available for either of these grassland areas. Cameron and Hamilton (4) obtained significantly higher beef gains from animals grazing on dikeland pasture compared to those grazing on upland. Much more information is required for efficient forage production.

Many experiments concerning height and frequency of clipping have been conducted on grassland crops. Frequency of cutting has been related to stage of growth, crop use and time intervals between harvests. Wagner (13), clipping mixtures to two stubble heights, found that the treatments that led to the highest total forage production were superior as to distribution of production throughout the season, prevention of weed encroachment, and maintenance of a satisfactory grass legume balance. Other workers (2, 3, 7, 8, 12), working with various crops and mixtures clipped to stubble heights of 1 to 4 inches, found that more yield was obtained from the closer clipped plots. Cook *et al.* (5) found that more forage was produced on crested wheat grass plots clipped to a 3-inch stubble height than on those clipped to a 1-inch stubble height. Kennedy (11), studying the effects of various clipping treatments on a blue grass - white clover sward, noted that to obtain a high yield of digestible organic matter it was necessary to cut the herbage periodically to a height of 1 inch. Juska *et al.* (10),

[1]Contribution No. 111, Cereal and Forage Division, Research Branch, Canada Department of Agriculture, Nappan, N.S.

working with lawn grasses, found that high nitrogen and low cutting treatments inhibited root and rhizome production. Most workers (1, 2, 5, 6, 8, 9) agree that increased frequency of clipping decreased the total forage production for the season.

This paper presents the results of an experiment undertaken to compare forage production on upland and dikeland under different clipping intensities, and to determine the influence of these treatments on the botanical composition of the sward.

MATERIALS AND METHODS

A mixture of 3 pounds timothy, 6 pounds orchard grass, 4 pounds red clover, 4 pounds alfalfa, and 1 pound Ladino clover was seeded in 1956 on separate areas of Acadia soil type (dikeland) and Nappan soil type (upland) on the Experimental Farm, Nappan, Nova Scotia. Acadia soil type is a tidal deposited silty loam, relatively well-drained and characterized by slow lateral water movement with a moderately high water table. Nappan soil type is a heavy clay loam of glacial origin, poorly drained and severely leached. Both soils were slightly acid, the Acadia having a pH of 6.8 and the Nappan a pH of 6.7.

Prior to seeding, a general fertilizer application was made at the rate of 500 pounds per acre of 6-12-12. In the spring of the third harvest year a subsequent application of 6-12-12 at 300 pounds per acre was supplied.

The experiment was set up as a split, split plot with four replications on each soil type. Soil types made up the main plots, stubble heights the major sub-plots, and frequency of cut the secondary sub-plots. Plot size

TABLE 1. — FORAGE PRODUCTION IN POUNDS PER ACRE OF DRY MATTER ON TWO SOILS UNDER VARIOUS CUTTING TREATMENTS FOR 3 YEARS AT NAPPAN, NOVA SCOTIA

Year	1957			1958			1959			3-yr. av.		
Stubble height	1¾″	3″	Av.	1¾″	3″	Av.	1¾″	3″	Av.	1¾″	3″	Av.
Upland Soil												
Frequency												
3-week	2657	2182	2420	3883	3531	3707	2196	1753	1975	2910	2489	2700
4-week	3652	2992	3322	5155	4575	4865	2580	2123	2352	3796	3230	3513
8-week	6211	5517	5864	7284	6760	7022	5693	4578	5136	6394	5618	6006
Av.	4173	3564	3868	5441	4955	5198	3490	2818	3154	4367	3779	4073
Dikeland Soil												
3-week	3625	3177	3401	4881	4694	4788	2225	1969	2097	3827	3280	3554
4-week	5000	4524	4762	5715	5624	5670	2870	2402	2636	4528	4184	4356
8-week	7081	6400	6741	8254	7839	8047	5592	4854	5223	6975	6364	6670
Av.	5235	4700	4968	6283	6052	6168	3562	3075	3319	5110	4609	4860
L.S.D. P = .05												
Soils		576			346			N.S.			382	
Heights		181			201			149			156	
Frequency		294			157			200			157	

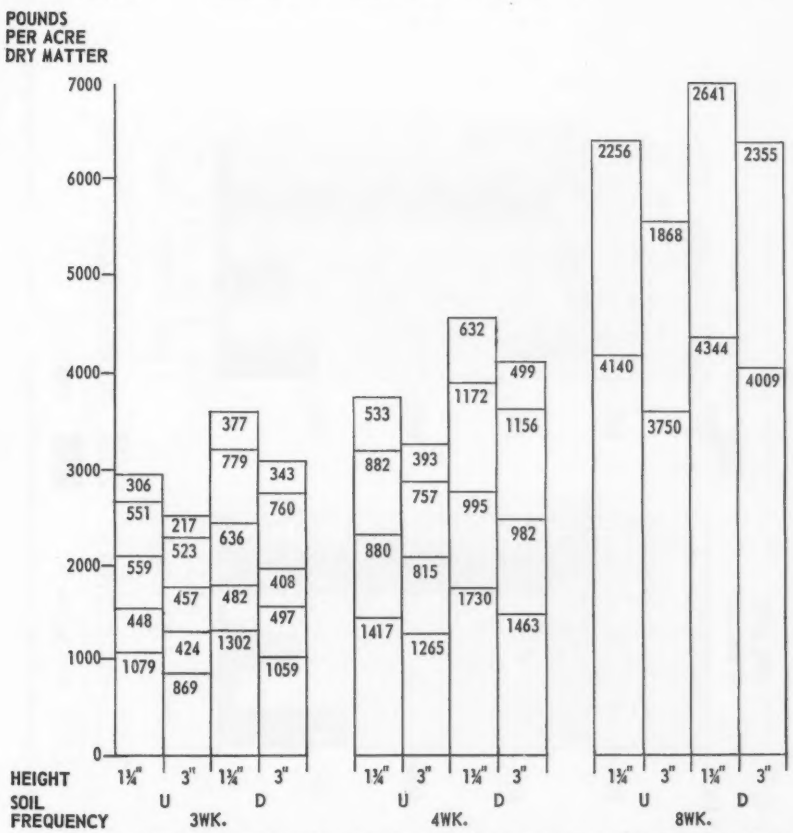

FIGURE 1. Average annual production by cuts of dry matter produced with various cutting treatments on dikeland and upland, Nappan, N.S., 1957-59 inclusive.

was 6 by 30 feet. The harvested sample consisted of a mower width through the centre of each plot. From this chopped material two 500-gram samples were taken from each plot for dry matter determination. Data were obtained for three harvest seasons following the year of seeding.

The two stubble heights compared were 1¾ and 3 inches, obtained by using the Gravely mower without riders and with riders respectively. The 3-week frequency plots were cut five times each year, the 4-week frequency plots four times and the 8-week frequency plots twice. Cutting commenced the first week in June and ended the first week in September.

Inclined point quadrat readings were recorded as hits per species for 30 needles per plot. These readings were taken each spring before any plots were clipped and again each fall approximately 3 weeks after the last clipping date. The results of each survey were averaged as per cent contribution by species to determine the species response to the various clipping frequencies. Weed invasion was also recorded.

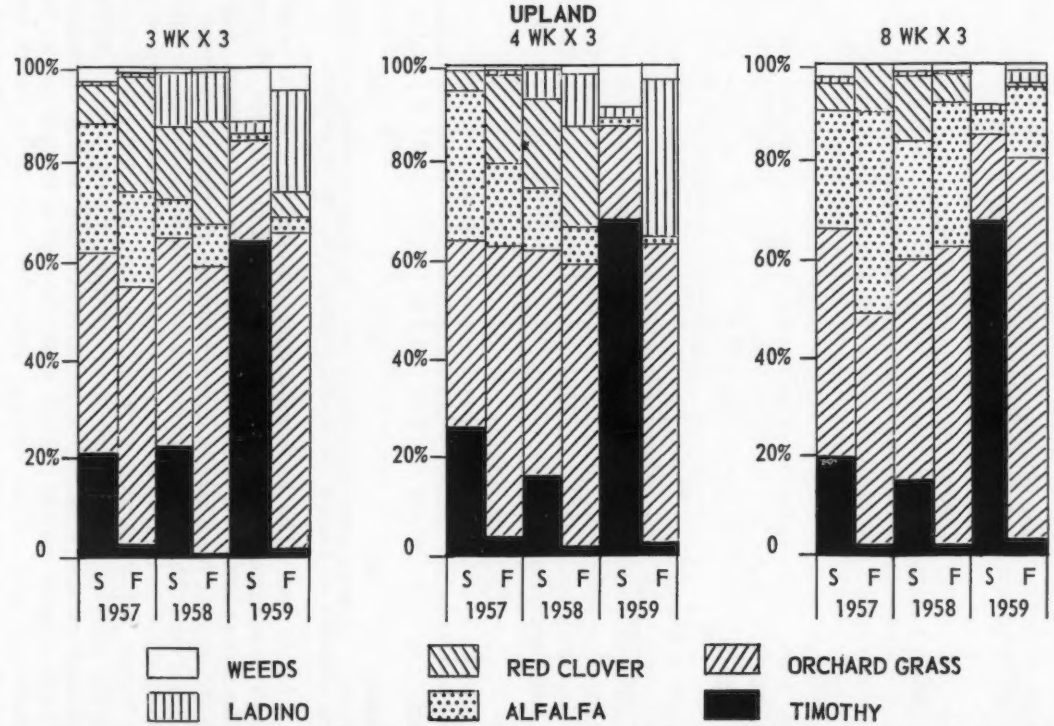

FIGURE 2. Percentage contribution by species of a forage mixture on upland cut to a stubble height of 3 inches at three frequencies.

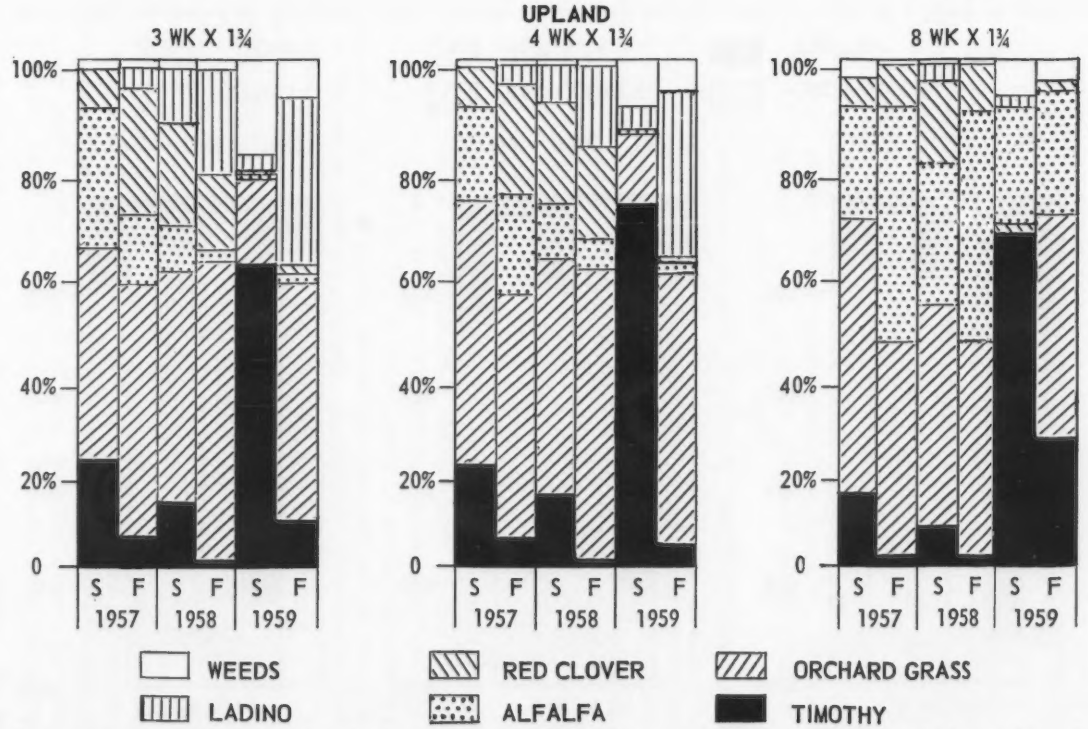

FIGURE 3. Percentage contribution by species of a forage mixture on upland cut to a stubble height of 1¾ inches at three frequencies.

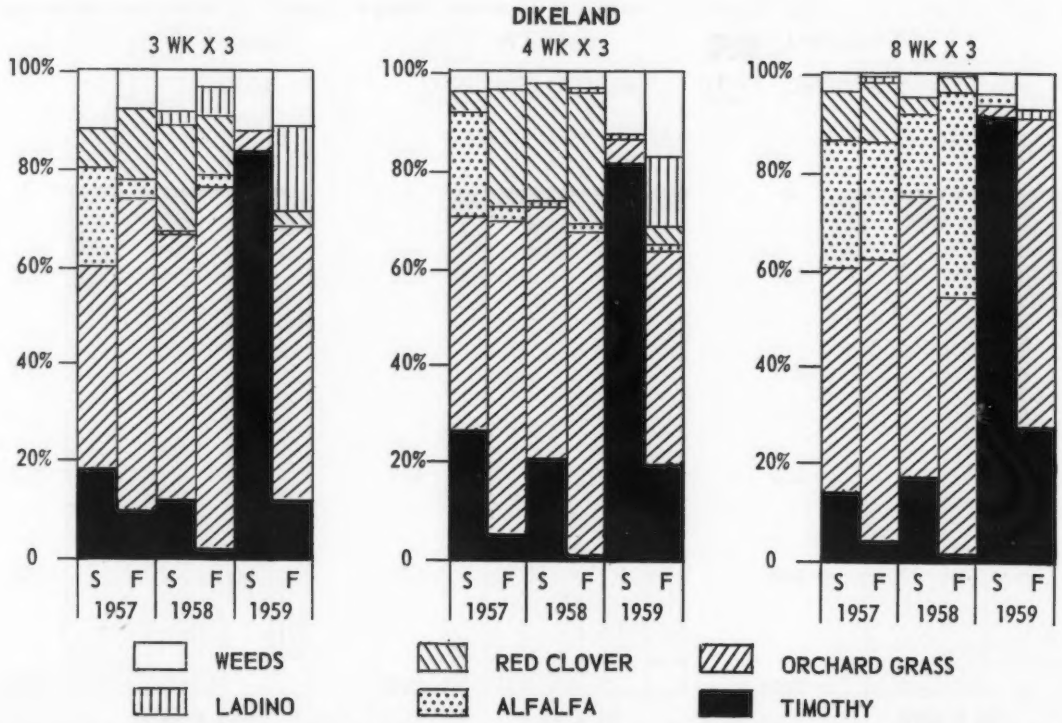

FIGURE 4. Percentage contribution by species of a forage mixture on dikeland cut to a stubble height of 3 inches at three frequencies.

EXPERIMENTAL RESULTS AND DISCUSSION

Yield

The average yield of each treatment in each year is shown in Table 1 as pounds of dry matter per acre. Included in this table also are the average yields for the 3-year period and the least significant differences required for all comparisons.

Significant differences were noted for every comparison, except that between the two soil types in the third harvest year. For every cutting treatment greater yields were obtained on dikeland than on upland. As an average of all treatments the dikeland yield was 20 per cent greater than the upland. In every case cutting at the 8-week interval resulted in considerably greater total yield than did cutting more frequently. Similarly, higher yields were obtained from plots clipped at 4- rather than 3-week intervals; this agrees with other reports (1, 2, 5, 6, 8, 9). Plots clipped to a stubble height of 1¾ inches always produced greater yields of dry matter than plots clipped to a 3-inch height.

Seasonal distribution of yield is generally considered almost equal in importance to actual total yield. The above results may be also observed in Figure 1, which presents graphically the 3-year average production by harvest periods, by heights of clipping and by soil types. In addition, it may be noted that the seasonal distribution of yield for all treatments was much more uniform on dikeland than on upland. The similarity of the differences in production for the various treatments on the two soils indicates that similar management practices are justified for the two areas.

Species Contribution

The percentage contribution of the species, in the spring and fall of each harvest year, as determined from inclined point quadrat sampling, is shown in Figures 2 and 3 for the two stubble heights on upland and Figures 4 and 5 for the two stubble heights on dikeland. A striking element of these data is the similarity of the species complex not only on the two soils but also for the various cutting treatments. Accordingly, they may be discussed conveniently by species.

Timothy – Timothy contribution varied greatly between spring and fall readings and averaged considerably less than orchard grass. Timothy declined greatly during the second summer and recovered to become the dominant species in every treatment in the spring of the third year. Height of cutting apparently had no effect on this species.

Orchard Grass – Orchard grass was much more uniform in production than was timothy in all comparisons. In most of the seasonal surveys orchard grass made up more than 40 per cent of the growth. It showed considerable reduction in the spring of the third year, probably due to the previous severe winter, but it made good recovery by the time of the fall survey.

Alfalfa – Alfalfa made up 20 per cent or more of the forage in the spring of the first year. It declined gradually on upland and rather quickly on dikeland under the more intense clipping frequencies. Under these treatments it was practically eliminated by the spring of the third year. Per-

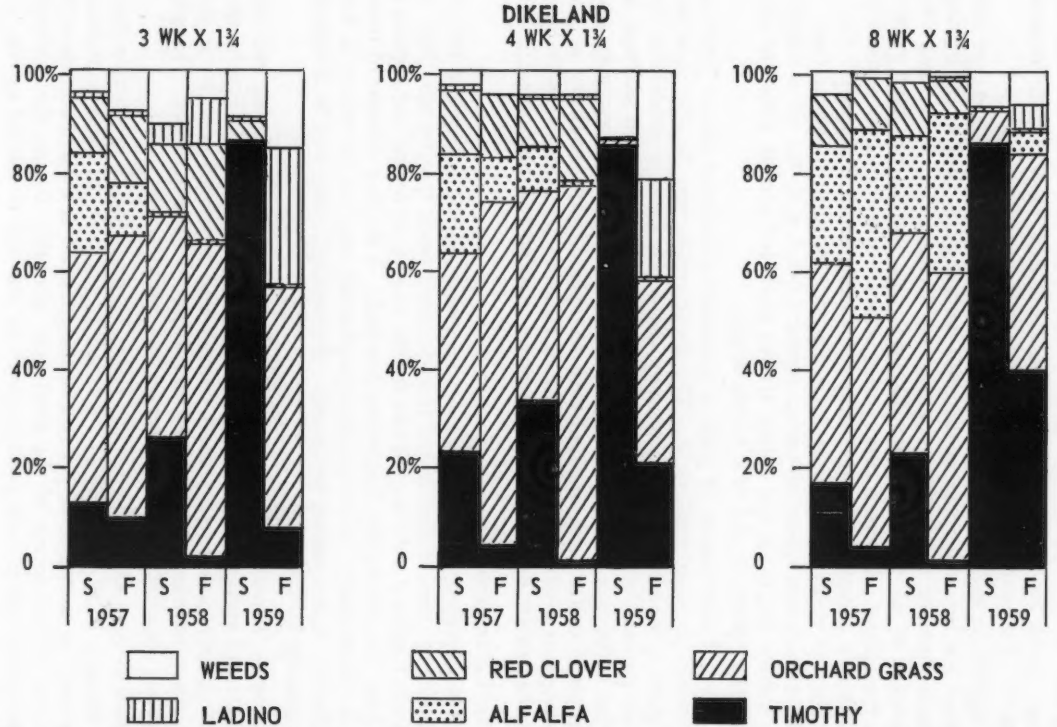

FIGURE 5. Percentage contribution by species of a forage mixture on dikeland cut to a stubble height of 1¾ inches at three frequencies.

sistence was best at the 8-week clipping, most notably on upland and at the closer stubble height. The severe winter of 1958-59 was largely responsible for much of the loss in alfalfa. Alfalfa does not tolerate the high water table characteristic of dikeland soil for more than two or three seasons.

Red Clover – Red clover contributed slightly less than 10 per cent of the forage in the spring of the first year. It remained at about this level in the 8-week frequency through the fall of the second harvest year and then practically disappeared. For the 3- and 4-week frequencies the proportion increased to about 20 per cent for 2 years before being eliminated in the third year. Red clover showed considerable similarity between spring and fall readings.

Ladino Clover – The productivity of Ladino clover increased each year of the test. It was particularly favoured by the more frequent and closer clipping. Its performance was poorest at the 8-week frequency and it was considerably poorer on dikeland than upland.

Weeds – Weed invasion was very minor in all treatments. Weed amounts were slightly more apparent on dikeland but showed little difference between frequencies of clipping or heights of clipping. In the third-year survey, weeds constituted a larger proportion of the growth than in the previous years.

The seeded species produced practically all of the plant growth throughout the 3 years of the test. The performance of orchard grass, alfalfa, and Ladino clover merits special attention, as these species are not commonly used in forage mixtures in the Maritime Provinces. This is especially important, due to the highly variable response of timothy and the short-term value of red clover.

SUMMARY

Forage yield was 20 per cent greater on dikeland and seasonal distribution was better on dikeland than on the upland area with which it was compared. Production on both soils was highest when plots were clipped at 8-week intervals and at 1¾ inch stubble height with yields decreasing as frequency of cut increased. Timothy contribution fluctuated widely between high spring values and low fall values each year and extremely so in the third year. Orchard grass made a regular contribution of about 40 per cent each year. Alfalfa, though contributing reasonably well initially, failed to persist into the third year under more intense clipping, particularly on dikeland. Ladino clover increased in relative value during the 3 years, performing better on upland and under more intense use.

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VERTICILLIUM WILT OF POTATO IN RELATION TO VASCULAR INFECTION OF THE TUBER[1]

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Canada Department of Agriculture, Charlottetown, Prince Edward Island

[Received for publication April 6, 1961]

ABSTRACT

Several potato varieties, differing in their susceptibility to *Verticillium albo-atrum* Reinke & Berthold, differed similarly in the degree to which the fungus invaded the vascular tissue of stems and tubers. Surface-sterilized, internally infected seed pieces consistently gave rise to a lower incidence of wilt than healthy seed pieces of the same variety carrying surface-borne inoculum only. In all the varieties tested, a preponderance of tubers produced by wilted plants were apparently free from vascular infection, and those that were infected more often than not gave rise to wilt-free plants.

INTRODUCTION

Verticillium albo-atrum Reinke & Berthold incites wilt in the potato, either through invasion by externally borne inoculum or through a permeation of new growth by internal mycelium present in the vascular system of seed tubers. It has been reported that varieties differ in the extent to which the fungus is carried as mycelium in the tubers from wilted plants, and that the chief economic loss occurs in those varieties that are susceptible to both internal tuber infection and to external invasion by the fungus (1). A study of the effect of surface-borne tuber inoculum in one variety, Irish Cobbler, showed that such inoculum was more important in inciting disease than was the inoculum carried internally as vascular mycelium. In line with this finding, it was found that a high degree of wilt control in Irish Cobbler was possible by a seed treatment that destroyed surface-borne elements of *Verticillium* (2).

The purpose of the present paper is to present the results of a study on the relative importance of surface-borne and of internal, vascular inoculum in the perpetuation of field wilt from year to year. Several varieties, known to differ in susceptibility, were used.

MATERIALS AND METHODS

The varieties used in this study were chosen from a group of 15 that were assessed for wilt resistance beforehand. This was done in two ways: first, by an inoculation of seed pieces from healthy tubers through dipping in conidial suspensions of the pathogen, followed by observation of wilt development in the field; second, by using tubers saved from wilted plants as seed, treating with a fungicide to destroy surface inoculum and then observing wilt development on land where Verticillium wilt had not previously been encountered. Eight varieties, representative of the range of wilt susceptibility observed, were chosen. These, with wilt indexes, are listed in Table 1.

These data, on which the selection of varieties was based, indicate that varieties, such as Irish Cobbler and Kennebec, that are susceptible to wilt induced by external inoculum are also more subject to wilt arising from internally borne mycelium.

[1]Contribution No. 103, Experimental Farm, Canada Department of Agriculture, Charlottetown, P.E.I.
[2]Deceased.

TABLE 1.—THE RELATIVE SUSCEPTIBILITY OF EIGHT POTATO VARIETIES TO VERTICILLIUM WILT

Variety	Wilt index[1]	
	Surface inoculum	Internal inoculum
Irish Cobbler	50.8	23.5
Kennebec	47.2	12.4
Delus	42.2	10.9
Sebago	39.4	10.1
Boone	33.1	10.0
Saco	31.2	6.8
Fundy	19.7	6.8
Green Mountain	17.6	0
L.S.D. (P = 0.05)	8.8	8.6

[1]Based on 160 plants per treatment in a 4-replicated design, with percentage data converted to degrees of an angle as given by Snedecor (3)

Verticillium was isolated from host tissue by surface sterilizing pieces of stem, or, in the case of tubers, excising stem-end pieces containing some vascular elements. The sections were dipped in 2 per cent sodium hypochlorite for about 1 minute and then plated directly on potato dextrose agar acidified to pH 4.0 with 25 per cent lactic acid. In all cases where the comparative recovery of the pathogen was being studied, second isolations were attempted when the first had proved negative.

Experimental lots of internally infected tubers of the several varieties were selected in the laboratory in the year previous to test. This was done by attempting to isolate *Verticillium* from the vascular tissue of large numbers of tubers collected from wilted plants in the autumn. Tubers found to be infected were held in warm storage until cuts were suberized, then kept at 38°-44°F. until planting time the following year. *Verticillium*-free tubers, hereafter designated 'healthy', were obtained from potato stocks in which field wilt had not been encountered.

Surface inoculation was always carried out by dipping seed pieces immediately before planting in heavy spore suspensions of the fungus grown on sterilized wheat kernels or on potato dextrose agar.

Tubers to be surface-cleansed before planting were separately washed and brushed three times under running water. Surface treatment was carried out by dipping whole tubers in standard solutions of Semesan Bel (hydroxymercurinitrophenol 12.5 per cent, hydroxymercurichlorophenol 3.8 per cent) and allowing them to drain dry before cutting to seed pieces.

EXPERIMENTAL RESULTS

Infected tubers of the eight varieties were divided into three lots. One lot was washed, a second washed and treated, and the third lot surface inoculated. A control lot of healthy, inoculated seed was included and all lots then planted and observed for wilt development. The results (Table 2) show that, for all varieties, external inoculum was more effective than

TABLE 2. — THE EFFECT OF SURFACE INOCULATION AND OF TREATMENT OF HEALTHY AND NATURALLY INFECTED SEED PIECES ON THE INCIDENCE OF FIELD WILT IN SEVERAL POTATO VARIETIES

Variety	Percentage of plants wilted[1]			
	Healthy inoculated seed	Infected washed seed	Infected washed and treated seed	Infected inoculated seed
Irish Cobbler	62.0	16.0	24.0	60.0
Kennebec	61.2	8.7	7.5	51.2
Delus	52.5	11.2	1.2	47.5
Sebago	48.7	13.7	6.2	36.0
Boone	46.2	16.2	1.2	53.7
Saco	46.0	12.0	6.0	48.0
Fundy	34.0	6.0	4.0	50.0
Green Mountain	16.0	2.0	0	10.0

[1]Based on 80 plants per treatment for the varieties Sebago, Kennebec, Delus and Boone and 50 plants for the varieties Irish Cobbler, Saco, Fundy and Green Mountain

TABLE 3. — THE EXTENT OF VASCULAR TUBER INFECTION AND ITS EFFECT IN INCITING WILT IN FIVE POTATO VARIETIES

Variety[1]	Per cent internally infected tubers in harvests from wilted plants	Per cent tubers internally infected giving rise to wilted plants[2]
Irish Cobbler (156)	27.5	28.6
Kennebec (266)	30.4	22.5
Sebago (179)	17.3	20.0
Boone (160)	41.2	12.1
Green Mountain (263)	6.1	0

[1]Figures in parentheses are number of tubers from which isolations were attempted.
[2]Infected tubers were surface cleansed by washing and treating prior to cutting into seed pieces.

internal mycelium in inciting wilt. Some varieties, however, appear to be more capable than others of transmitting the disease through infected tubers.

Further data on transmission through vascular infection of the tuber were obtained in a trial with five varieties in which infected tuber lots of each were washed and treated. The data on the extent of seed tuber infection and the resultant field wilt (Table 3) show that less than one-third of the tubers produced by wilted plants were internally infected and, of these, generally less than one-quarter gave rise to wilted plants.

It has previously been reported that potato plants may act as symptomless carriers of *Verticillium* (2). Varietal reaction in this respect was studied in two growing seasons by attempting to isolate the fungus at midseason from apparently healthy potato stems of plants grown from vascularly infected seed pieces. For each stem serial sections were plated from the first 6 inches above ground level and a positive recording made if *Verticillium* grew from any of the stem pieces. Percentages of symptomless, but infected, stems found are given in Table 4. It is apparent that the

TABLE 4. — THE DEGREE OF INVASION BY *VERTICILLIUM* IN SYMPTOMLESS PLANTS OF FIVE POTATO VARIETIES GROWN FROM SEED PIECES WITH VASCULAR INFECTION

Variety	Percentage of wilt-free stems found to be infected[1]	
	Trial A	Trial B
Irish Cobbler	47.5	51.5
Kennebec	25.0	32.3
Sebago	27.5	20.6
Boone	17.5	26.5
Green Mountain	5.0	19.1

[1]Based on 40 stems in Trial A and 68 stems in Trial B for each variety

fungus was present in a high percentage of non-wilted plants of all varieties and that the most susceptible varieties were most heavily invaded. In one test-year a comparison was made of the relative fungal invasion of symptomless stalks of five varieties when the infected seed pieces had been either washed or washed and then surface-treated. The percentage recovery of *Verticillium* in 150 isolation attempts with each variety was as follows:

Treatment	Variety				
	Kennebec	Irish Cobbler	Boone	Sebago	Green Mountain
Seed washed	51.3	31.3	33.3	22.0	7.3
Seed washed and treated	20.6	32.6	12.6	10.0	2.6

It is evident that the greater the surface cleansing action, the less the fungal invasion of the plant. Thus washing, followed by treatment with Semesan Bel, allowed the least infection of the stems, except in the variety Irish Cobbler. This is in accord with the finding (Tables 1, 2 and 3) that this variety transmits the disease from internally infected seed more readily than other varieties studied.

The apparent activity of the organic mercury fungicide, Semesan Bel, in suppressing vascular infection suggested that it might have chemotherapeutic activity. However, no such activity could be demonstrated in a bio-assay procedure. Irish Cobbler seed pieces were treated with Semesan Bel (100 p.p.m.) and embedded in damp, sterilized moss until sprouts of about 1 inch in length were produced. The epidermis was then peeled off and the sprouts sectioned into pieces 3 millimetres thick. These, together with checks from untreated seed pieces, were placed on nutrient assay agar seeded with standardized suspensions of *Bacillus subtilis**. No trace of inhibition was ever detected even though control series of filter paper assay

*Product of Difco Laboratories, Detroit, Mich.

discs saturated with solutions of Semesan Bel produced distinct inhibition zones when the chemical was used at concentrations ranging from 100 to 0.3 p.p.m.

DISCUSSION

Verticillium wilt of potato is spread by means of inoculum in the soil or on the surface of tubers, and by mycelium within the tubers. The relative importance of surface-borne inoculum, in contrast to vascular infection of seed tubers, has been reported in the variety Irish Cobbler (2), and is further supported by the results of this study. In all eight varieties tested a much higher incidence of wilt was obtained from non-infected, surface-inoculated seed than from infected, non-inoculated seed (Table 2). It is also notable that, in general, the high level of disease incited by surface-borne inoculum was not increased by the presence of vascular mycelium in the seed. Others (4), working with potato wilt but using a microsclerotial form of *Verticillium* in contrast to the non-sclerotial form used by the authors, have reported that high levels of external soil inoculum were more effective than low levels in inciting foliar symptoms. The relatively minor extent of wilt produced by internal mycelium alone may possibly be due to the scanty inoculum load in contrast to the large amount of inoculum present following surface inoculation. It has been shown (2) that natural surface inoculum may be present in great quantity in an infected crop.

The degree of susceptibility of varieties to wilt is reflected in the ease with which the fungus invades tubers and stalks. An assessment of the extent of tuber infection in stocks from wilted plants (Table 3) and of the progress of the organism in symptomless plants grown from infected tubers (Table 4) showed that the extent of fungal invasion was a good index of varietal susceptibility to the wilt phase of the disease. Susceptible varieties, such as Irish Cobbler and Kennebec, were generally more heavily invaded than less susceptible varieties such as Boone and Green Mountain. It was of interest in the isolation work that varieties most susceptible to *Verticillium* also quite frequently harboured other, non-pathogenic soil fungi. *Verticillium* was obtained most often in the pure state from the variety Boone.

It is concluded from this work that, although vascular invasion is necessary for the development of wilt, it is apparently of minor importance in the perpetuation of the disease. It follows that sanitation and seed treatment measures designed to lessen external inoculum are of prime importance in the control of Verticillium wilt of potato.

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance of C. T. Mullin in carrying out this study.

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EFFECT OF SULPHUR TREATMENTS ON YIELD AND NITROGEN AND SULPHUR CONTENT OF ALFALFA GROWN ON SULPHUR-DEFICIENT AND SULPHUR-SUFFICIENT GREY WOODED SOILS

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[Received for publication February 24, 1961]

ABSTRACT

Sulphur applied in the elemental form, or as sodium or calcium sulphate, on Grey Wooded soils, increased yields of alfalfa on sulphur-deficient Loon River loam, but not on sulphur-sufficient Garrick loam. The quantity of sulphate-sulphur was extremely low in the alfalfa grown on untreated Loon River loam and increased markedly as a result of the application of sulphur to the soil. Although herbage from untreated areas of the sulphur-deficient soil contained a slightly higher per cent nitrogen in 1955 than that grown on the sulphur-sufficient soil, sulphur treatment increased nitrogen content and yield of nitrogen on the sulphur-deficient soil. The relative abundance of nitrogen in the herbage grown on the check areas of the deficient soil suggests that the main effect of sulphur was not on root nodulation or nitrogen supply, but rather on nitrogen assimilation and other physiological functions of the sulphur-containing compounds within the plant.

INTRODUCTION

In the spring of 1955 a study was initiated to determine the effect of sulphur treatments on the yield and nitrogen and sulphur content of alfalfa when grown on a sulphur-deficient and a sulphur-sufficient Grey Wooded soil. One of the objectives of this study was to characterize the effect of sulphur treatments on the crop in an effort to shed light on the nature of the response, and to assess the value of plant analysis in predicting such response.

Crop response to applied sulphur has long been recognized in many parts of the world. Hart and Peterson (8), Miller (14), Eaton (4) and Lipman (11) all reported work during the period 1911 to 1924. The need for applied sulphur was recognized in Canada in 1927 (24).

Alway (1) gave the critical sulphur content of alfalfa as 0.20 per cent. However, Bentley *et al.* (2) reported sulphur contents of 0.17 per cent in first-cut legumes and 0.14 per cent in second-cut legumes grown on soils not responsive to applied sulphur. As long ago as 1914, Peterson (19), and later Shedd (20) and Eaton (5), found that the soluble sulphate fraction of the plant sulphur was the only fraction to be markedly influenced by sulphur fertilization.

In a review of sulphur nutrition Gilbert (6) stated that sulphur occurs in plant tissues chiefly in proteins, volatile compounds and sulphates. Meyer and Anderson (13) stated that sulphur is a constituent of the important plant hormones thiamine and biotin, and that amino acids and other nitrogen-containing compounds accumulate in the tissues of sulphur-deficient plants. Neilands *et al.* (16) stated that sulphur is a constituent of co-enzyme-A and α lipoic acid. Alfalfa grown on soils treated with sulphur was found by various workers (17, 18) to contain a higher percentage of

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protein than alfalfa grown on soils not so treated. Red clover grown under sulphur-deficient conditions was found by Tottingham (22) to have a limited protein-synthesizing ability and restricted growth.

According to Mehlich and Drake (12), the plant absorbs sulphur as the sulphate ion and part of it is reduced in the plant to become a constituent of the amino acids cystine and methionine. Sulphur serves also as a regulatory element in the synthesis of chlorophyll, the acceleration of root development, a redox system in respiration and in the activation of certain enzymes.

Thomas *et al.* (21) reported that alfalfa plants provided with high nitrogen and low sulphur supply had an appreciably increased nitrogen content, and that the excess nitrogen was soluble amide, amino and possibly nitrate-nitrogen.

MATERIALS AND METHODS

Three sulphur carriers, viz., elemental sulphur, gypsum and sodium sulphate (Glauber's Salt), were drilled into established stands of alfalfa on Loon River loam (sulphur-deficient) and Garrick loam (sulphur-sufficient) Grey Wooded soils in northern Saskatchewan. The two soils have been described by Moss and St. Arnaud (15). The materials were drilled to a half-inch depth on both soils in plots with dimensions of 6 by 30 feet. Each carrier was applied in the spring of 1955, before the commencement of active growth, to provide 30 and 60 pounds of sulphur per acre. Each sulphur treatment was replicated sixteen times at each location. The yield, sulphur and nitrogen content of alfalfa were determined in 1955 and 1956.

Yields were determined by standard procedures. In 1955 composite grab samples of the above-ground portions of the plant were ground for chemical analysis, while in 1956 young leaves were plucked from growing plants at harvest time. Total sulphur content of the herbage was determined by combining the oxidation method outlined by Ward and Johnston

TABLE 1. — THE EFFECT OF SULPHUR TREATMENTS ON THE YIELD OF ALFALFA

Treatment per acre applied in 1955	Pounds of dry matter produced per acre[1]					
	Loon River Loam			Garrick Loam		
	1955	1956	Average	1955	1956	Average
No treatment	1308	763	1036	2932	2642	2787
Sulphur (30S)[2]	1196	1035	1116	2944	2631	2788
Sulphur (60S)	1372	1226	1299	2966	2571	2769
$CaSO_4$ (30S)	1534	1371	1439	2943	2613	2778
$CaSO_4$ (60S)	1616	1467	1541	3030	2419	2725
Na_2SO_4 (30S)	1675	1412	1543	3004	2670	2837
Na_2SO_4 (60S)	1775	1453	1614	2909	2608	2758
L.S.D. (0.05)	139	88	88	—	—	—

[1]Average of 16 plot values each year
[2]Pounds of sulphur per acre provided by treatment

(23) and the turbidimetric method outlined by Chesnin and Yien (3). Sulphate-sulphur was extracted with 2 per cent acetic acid and determined by the turbidimetric method. 'Protein' sulphur was assessed by extracting the plant material with 5 per cent trichloroacetic acid (9). Total nitrogen was determined by the official Kjeldahl procedure, and protein nitrogen by the method outlined by Kolousek and Coulson (10).

RESULTS AND DISCUSSION

Alfalfa growth on Loon River loam was restricted by dry climatic conditions in both years, but was sufficient to exhibit marked response to treatment. The Garrick soil produced an average crop.

Yield of Dry Matter

The mean yields of dry matter of alfalfa grown with and without applied sulphur on the two soils are presented in Table 1.

Sulphur treatments effectively increased the yield of alfalfa on the deficient Loon River soil. In general, the higher rates of each carrier were more effective than the lower rates. Sodium sulphate was more effective in the year of application than gypsum. Gypsum was more effective than elemental sulphur, which failed to cause crop response in the year of application. This test indicates that response to sulphur was dependent on the availability of the sulphur in the carrier. Sulphur treatments had no significant effect on the yield of alfalfa grown on Garrick loam.

Sulphur in the Crop

The effects of sulphur treatments on the total sulphur and soluble sulphate-sulphur content of alfalfa are shown in Table 2.

TABLE 2. — THE EFFECT OF SULPHUR TREATMENTS ON THE SULPHUR CONTENT OF ALFALFA

Treatment per acre applied in 1955	Total sulphur[1]				Soluble sulphate sulphur[1]				Sulphur uptake by crop[1]	
	Loon River Loam		Garrick Loam		Loon River Loam		Garrick Loam		Loon River Loam 1955	Garrick Loam 1955
	1955	1956	1955	1956	1955	1956	1955	1956		
	%	%	%	%	%	%	%	%	lb./ac.	lb./ac.
No treatment	.17	.14	.23	.18	.01	.02	.13	.09	2.25	6.78
Sulphur (30S)	.17	.17	.24	.19	.01	.04	.15	.10	2.00	7.13
Sulphur (60S)	.20	.17	.25	.18	.03	.06	.14	.12	2.75	7.51
$CaSO_4$ (30S)	.25	.18	.26	.19	.11	.05	.16	.11	3.83	7.56
$CaSO_4$ (60S)	.26	.20	.26	.18	.14	.09	.18	.13	4.28	7.93
Na_2SO_4 (30S)	.27	.19	.25	.18	.12	.06	.16	.11	4.52	7.48
Na_2SO_4 (60S)	.29	.20	.27	.19	.16	.09	.17	.13	5.14	7.80
L.S.D. at 5 per cent level	.03	—	.03	—	*	*	*	*	0.66	0.93

[1]Average of 16 plot values each year
*Significant at the 1 per cent level, L.S.D.'s very small

The total sulphur content of the herbage was slightly increased without affecting the yield of dry matter when sulphur was applied to the non-responsive soil. However, on the responsive soil the yield increased generally in proportion to the increased level of sulphur in the herbage. The best yield on the responsive soil was obtained after the sulphur content of the herbage was well above the level in the herbage grown on the untreated non-responsive soil. In 1955, a content of 0.23 per cent apparently represented a sufficient supply of sulphur on the Garrick soil, while the best yield was obtained on the Loon River soil after the content was raised to 0.27 per cent. However, in 1956, when the crop leaves were sampled, the level of sulphur in the best-yielding Loon River plots was approximately equal to the level in the leaves sampled from the untreated plots on Garrick loam. In general, when composite samples of the crop were analysed, in 1955, the untreated Loon River soil produced herbage with a sulphur content well below the "critical" content of 0.20 per cent reported by Alway (1). However, if the 1956 data are considered, then the crop on each soil was in the "deficiency" range.

The soluble sulphate-sulphur content of the herbage was the clearest index of the sulphur-supplying status of these soils. The sulphate-sulphur content of the herbage on the better-yielding plots of the responsive soil approached the content of the check plots on the non-responsive soil. The sensitivity of this fraction as an index of sulphur supply is in agreement with the findings of other workers (5, 19, 20).

The sulphate-sulphur content of the herbage was studied for correlation with yield on all samples analysed for both soils. On Loon River loam the coefficients of correlation, +.36 in 1955 and +.65 in 1956, were significant at the 1 per cent level, while on Garrick loam all of the coefficients were negative, but non-significant.

TABLE 3. — THE EFFECT OF SULPHUR TREATMENTS ON THE NITROGEN CONTENT OF ALFALFA

Treatment per acre applied in 1955	Nitrogen[1]				Nitrogen uptake by crop[1]	
	Loon River Loam		Garrick Loam		Loon River Loam 1955	Garrick Loam 1955
	1955	1956	1955	1956		
	%	%	%	%	lb./ac.	lb./ac.
No treatment	3.02	2.34	2.92	2.80	39.9	85.1
Sulphur (30S)	2.94	2.61	2.86	2.86	35.3	83.8
Sulphur (60S)	3.15	2.70	2.93	2.85	43.3	87.1
$CaSO_4$ (30S)	3.21	2.69	2.97	2.81	49.4	86.7
$CaSO_4$ (60S)	3.23	2.93	2.89	2.80	52.4	87.6
Na_2SO_4 (30S)	3.27	2.87	2.88	2.78	54.9	86.0
Na_2SO_4 (60S)	3.23	2.80	3.03	2.88	57.5	87.8
L.S.D. (0.05)	0.12	0.15	—	—	6.9	—

[1]Average of 16 plot values

In the crop harvested, the pounds of sulphur were determined only for the 1955 crop because of the changed method of sampling in 1956. The small uptake of sulphur from the Loon River soil, in a crop that can effectively recover applied sulphur, is evidence of a quantitative deficiency of the nutrient. Alfalfa on the Loon River soil recovered more of the sulphur from the sulphate-sulphur treatments than it did on the Garrick soil and yet recovered less of the sulphur from the elemental sulphur. Elemental sulphur either failed to oxidize in the Loon River soil during the first year or did not become available to the crop on oxidation.

Nitrogen in the Crop

Data showing the effect of the sulphur treatments on the nitrogen content of the crop are presented in Table 3.

There was a marked increase in the per cent total nitrogen of alfalfa on the sulphur-deficient soil in both test years as a result of sulphur treatments. The elemental form of sulphur was somewhat less effective than the sulphate forms. Sodium sulphate tended to be more effective than gypsum. The treatments had no significant effect on the nitrogen content of alfalfa grown on Garrick loam.

The uptake of nitrogen, as in the case of sulphur, was calculated for the 1955 crop only. Sulphur treatments had a marked effect on the amount of nitrogen in alfalfa harvested from the Loon River soil. The gypsum and sodium sulphate treatments increased the uptake of nitrogen to a very significant extent, while elemental sulphur had less effect. None of the treatments had a significant effect on the uptake of nitrogen on Garrick loam.

The fact that both the percent nitrogen and the yield of nitrogen were increased in alfalfa grown on Loon River loam by the sulphate-sulphur treatments might indicate that the effect of the sulphur was exerted mainly through the root nodule bacteria and related to the nitrogen supply. However, the alfalfa grown on the untreated areas of Loon River loam contained 3.02 per cent nitrogen in 1955 as compared with 2.92 per cent in the untreated alfalfa on Garrick loam. The nitrogen supply in the untreated Loon River crop would thus appear to be quite adequate for a greater production of dry matter. It would seem that sulphur plays a role in the utilization as well as fixation of nitrogen. This would be expected since sulphur is a constituent of several important biological compounds, e.g., thiamine, biotin, co-enzyme-A, and α lipoic acid (13, 16).

Protein Sulphur and Nitrogen

Herbage samples from untreated plots and from plots treated with 60 pounds of elemental sulphur and 604 pounds of sodium sulphate were analysed for total and protein sulphur and protein nitrogen in 1955 (Table 4).

The data are limited and somewhat variable but do indicate that only about two-thirds of the nitrogen was present in the protein form as measured by the method used. A somewhat smaller portion of the sulphur was found

TABLE 4. — THE EFFECT OF SULPHUR TREATMENTS ON TOTAL AND PROTEIN SULPHUR AND NITROGEN IN ALFALFA, 1955

Treatment per acre applied in 1955	Protein sulphur[1]		Total sulphur[1]		Protein nitrogen[1]		Total nitrogen[1]	
	Loon River Loam	Garrick Loam	Loon River Loam	Garrick Loam	Loon River Loam	Garrick Loam	Loon River Loam	Garrick Loam
	%	%	%	%	%	%	%	%
No treatment	.07	.08	.17	.21	2.08	1.92	3.01	2.72
Elemental sulphur (60S)	.10	.07	.20	.24	2.02	2.19	3.13	2.98
Na_2SO_4 (60S)	.09	.04	.28	.24	2.29	1.92	3.32	2.97

[1]Average of 4 plot values

in the protein fraction. The Loon River loam crop contained a higher per cent of both total and protein nitrogen than the Garrick crop. In view of this, it is doubtful that the effect of sulphur on the nitrogen supply played an important part in increasing the yield of herbage. These results are in agreement with the findings of Tottingham (22) and Thomas *et al.* (21).

General

It seems that the low supply of sulphate-sulphur in the plants grown under deficient conditions limits the synthesis of organic sulphur compounds and thus growth. A minimum excess of sulphate-sulphur seems to be required in the plant to maintain normal growth. Such a relationship has been set forth by various workers (7).

ACKNOWLEDGEMENTS

The authors wish to thank W. B. Towill and K. E. Bowren of the Scott and Melfort Experimental Farms, respectively, for conducting much of the field aspects of this study, and J. H. A. Labelle, Analytical Chemistry Research Service, for carrying out the nitrogen determinations.

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SPECIES TRIALS WITH OIL PLANTS. I. CRAMBE[1]

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[Received for publication March 17, 1961]

ABSTRACT

Yields of seed of Crambe grown at Ottawa, Ontario, exceeded those of flax in comparative tests. Oil content averaged from 33 to 35 per cent and iodine number from 92.5 to 97.5. The oil is quite high in erucic acid. It has the following fatty acid composition—palmitic 1.7 per cent; stearic 1.0; oleic 16.7; linoleic 7.8; linolenic 6.9; eicosenoic 2.9; behenic 2.7, and erucic 55.7. The seed averaged 24 per cent protein and 15 per cent fibre. The meal contained 1.2 milligrams isothiocyanate and 5.1 milligrams thiooxazolidone per gram of oil-free meal.

INTRODUCTION

Linseed flax for many years was the only oilseed crop that Canada produced in sufficient quantity to satisfy domestic requirements and produce a surplus for foreign trade. During the Second World War sunflower and rape came into commercial production and the acreage in soybean was greatly expanded. Following the war, trade in oilseeds was unsettled and the introduction of margarine in 1948 created a greater demand for edible oils. A project was initiated to examine a wide range of oil-producing plants and study the possibilities of their production in Canada. A preliminary report on this work has already been published (8) and this paper presents more detailed data on Crambe grown at Ottawa, Ontario.

LITERATURE REVIEW

According to Gelin (3) *Crambe abyssinica* had been the object of a breeding program at Weibullsholm, Sweden, prior to 1949. He states that Crambe produced a yield of about 2,000 kilograms of seed per hectare with an oil content of 40 per cent. Bagge and Nordestgaard (1) report that Crambe in Denmark yielded 619 kilograms of oil per hectare with an iodine number of 94. In these trials Crambe proved less susceptible to attacks by pests and fungi than other cruciferous oil-plants, e.g., summer rape.

Kucerov and Kislinski (5) claim that Crambe in Voronezh Province (U.S.S.R.) yielded 2,770 kilograms per hectare on a 5-year average. The seed contained 53 per cent oil with an iodine number fluctuating between 86 and 97. Yield trials in Kharkov Province (50°N. 37°E.) indicated the best sowing date to be in late April; but in Bashkir Province (55°N. 55°E.) the highest yields were obtained by sowing in the first half of May. Crambe was found to be resistant to cold and drought and also resistant to nearly all diseases and insects which affect other Cruciferae. Sharapov (9) states that, on the basis of existing experimental studies, Crambe can be recommended for large-scale production. High seed yield, high oil content and ease of cultivation make it suitable for light soil zones of the central and northern regions of U.S.S.R.

Krzymanski (4) made a chemical examination of the seeds, oil and cake. Crambe seed without the seed coat had a higher fat content than either spring or winter rape and differed but slightly in chemical properties

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from rapeseed oil. The seed cake obtained from husked seed contained up to 50 per cent protein and only a small amount of mustard oil and therefore could be used without any limitations as a possible feed concentrate.

Niewiadomski and co-workers (7) determined the fatty acid composition of the oil from Crambe, using molecular distillation and paper chromatography, as follows: myristic 4.7; palmitic 9.7; arachidic 2.7; behenic 0.1; oleic 18.7; linoleic 12.7, and erucic acid 51.4 per cent. Mazzani (6) introduced Crambe from the Weibullsholm Institute to Venezuela and described the growth of the plant there. It matured in 93 days, producing seed with 42.8 per cent oil with an iodine number of 84.3.

DESCRIPTION

The plant is an annual, erect with numerous branches, varying in height from 28-36 inches, depending upon the season. Leaves are large and pinnately-lobed. Flowers are white, numerous, very small and in extensive corymbose racemes. The fruit is spherical, single and indehiscent and varies in weight from 7.0 to 7.5 grams per thousand, with a husk 14 to 20 per cent by weight. The seed is single, light to olive brown in colour, spherical, 1 to 2 millimetres in diameter, and weighs 6.2 to 6.7 grams per thousand kernels. The single seed per fruit distinguishes it from rapeseed. The adherence of the husk facilitates its separation from many other small seeds. The plants are free from disease and insect pests at Ottawa. During the spring of 1956, when flea beetles were numerous on other introductions of Cruciferae, Crambe remained undamaged (8).

EXPERIMENTAL RESULTS

Thirty introductions have been received from several European countries. The yield per acre, oil content and iodine number of three of those received in 1954 are given in Table 1 in comparison with Rocket flax. They were seeded at Ottawa in quadruplicate rod-row plots in rows 1 foot apart in early May and matured in 93-109 days. All introductions greatly exceeded the seed yield of Rocket flax. The 1959 crop was affected by drought until the first of August, but Crambe withstood this adversity better than flax. The oil content of the unhusked seed of Crambe was generally less than that of Rocket flax. However, taking the seed yield into consideration, the return of oil per acre was higher with Crambe in every instance. The three introductions of Crambe varied in yield per acre, oil content and iodine number with season. However, C.D. 6619 averaged higher in all three characters.

TABLE 1. — AVERAGE YIELD PER ACRE IN POUNDS, OIL CONTENT AND IODINE NUMBER OF CRAMBE GROWN AT OTTAWA, ONTARIO, 1957 TO 1959

Strain	Origin	Yield/acre	Oil content	Iodine number
		lb.	%	(Wijs)
C.D. 6090	Poland	2400	33.7	94.5
C.D. 6470	U.S.S.R.	2173	33.2	92.5
C.D. 6619	Sweden	2411	35.4	97.5
Rocket flax	—	1154	37.8	186.0

TABLE 2. — FATTY ACID COMPOSITION OF CRAMBE IN COMPARISON WITH RAPE BY GLPC[1]

Fatty acids	Crambe C.D. 6090	Golden rape	Arlo rape
	Per cent of total fatty acids		
Myristic	0.1	—	—
Palmitic	1.7	3.5	2.7
Palmitoleic	0.3	0.3	0.2
Stearic	1.0	1.3	1.0
Oleic	16.7	18.7	26.8
Linoleic	7.8	15.0	17.2
Linolenic	6.9	7.0	9.4
Arachidic	1.3	0.5	0.6
Eicosenoic	2.9	11.0	10.6
Behenic	2.7	0.3	0.2
Erucic	55.7	42.4	31.3
Lignoceric	2.9	—	—

[1]Gas liquid phase chromatography analysis, using 6 foot by ¼ inch O.D. copper column, filled with 60-80 mesh acid washed C-22 fire brick coated with succinate-1, 4 butanediol polyester in the ratio of 6:1 W/W. Unit operated at 210°C., helium flow-rate 60 ml. per minute, injector temperature 240°C., bridge current 200 mc., recorder 2.5 mv.

TABLE 3. — ANALYSES OF CRAMBE AND RAPESEED FOR ISOTHIOCYANATE AND THIOOXAZOLIDONE
(expressed as milligrams per gram of fat-free meal)

Variety	Isothiocyanate	Thiooxazolidone
Rape — Arlo	4.88	1.33
Golden	4.33	5.31
Crambe — C.D. 6090	1.21	5.13

The fatty acid composition determined at the Prairie Regional Laboratory, Saskatoon, Saskatchewan, for Crambe and the Golden and Arlo varieties of rapeseed is given in Table 2. These analyses were determined by gas-liquid chromatography (2) using a butanediolsuccinate polyester liquid phase. In comparison with rape, Crambe is low in linoleic and eicosenoic but higher in erucic acid content.

The seed of Crambe contains 22 to 25 per cent protein. It should, therefore, produce a meal comparing favourably with linseed meal which is usually higher in protein than rapeseed meal. However, the seed also contains about 16 per cent fibre which is higher than either linseed or rapeseed meal. This is due to the adherence of the husk to the seed which, as already stated, makes up about 14 to 20 per cent of the weight of the seed samples. Some experimental work indicates that rapeseed meal contains toxic factors which affect the growth of some species of animals. Two of these factors are considered to be isothiocyanate and thiooxazolidone. The average of these constituents in rapeseed expressed as milligrams per gram of fat-free meal, as reported by Wetter and Craig (10), is given in Table 3 in comparison with Crambe C.D. 6090. It will be noted that Crambe averages 1.1 to 1.3 milligrams of isothiocyanate per gram which is about a quarter that of rapeseed meal. The thiooxazolidone content, on the other hand,

is comparable to that of Golden rape and other varieties of the *Brassica napus* type. However, since Crambe is lower in isothiocyanate than rapeseed, it might be expected to produce a meal which could serve in livestock rations as well as rapeseed.

CONCLUSION

Crambe is an oilseed which appears worthy of further consideration. It can be handled with the same seeding and harvesting equipment as that used for cereals and, therefore, costs of production are very similar. Crambe has exceeded flax in yield per acre, but has a slightly lower oil content. The oil is similar to rapeseed oil, except that it is much higher in erucic acid. The lines tested show variation in yield and oil content and would suggest that, with a systematic plant breeding program, much could be done to produce a plant of improved agronomic and commercial value.

ACKNOWLEDGEMENT

The authors wish to thank L. R. Wetter, Prairie Regional Laboratory, Saskatoon, Saskatchewan, for the analyses of isothiocyanate and thiooxazolidone content, and the Analytical Chemistry Research Service, Ottawa, Ontario, for the oil content and iodine number of Crambe samples.

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NUTRIENT PRESERVATION AND ACCEPTABILITY OF FORAGES ENSILED WITH SODIUM METABISULPHITE AT VARYING LEVELS OF COMPACTION AND DRY MATTER

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[Received for publication December 19, 1960]

ABSTRACT

Sodium metabisulphite treatments did not reduce ensiling losses in mixed forages of various dry matter percentages at different densities.

The quality and odour of low-density, low-dry matter silages were improved, but those of high-density silages were unaffected.

Bisulphite increased the acceptability to heifers of forages of low to medium dry matter ensiled at medium density. Bisulphite did not increase the acceptability of low-dry matter forages ensiled at low density.

INTRODUCTION

Additives and conditioners have long been used as aids in reducing storage losses and improving the feeding quality of silage crops. Recently, the use of sodium metabisulphite (hereafter called bisulphite) has been recommended in the United States for such purposes.

Gordon *et al.* (6) showed that treatment of unwilted mixed forages with bisulphite reduced dry matter losses and increased palatability and digestibility. However, with wilted forages there was no saving of dry matter. Bratzler *et al.* (3) reported reduced losses of nutrients, particularly sugars and starches, and improvement of palatability and feeding value when bisulphite was used. Allred *et al.* (1) reported that the use of bisulphite had little effect on silage made from a red clover mixture; it gave only slightly less loss. They concluded that the savings in dry matter were insignificant. McCone and Olson (7) found that the addition of bisulphite had no beneficial effect in preventing losses in the stack-type silo. The experiment cited above involved unreplicated treatments of relatively large tonnages of crop. With such tests, uniformity of the ensiled crops is difficult to attain, storage losses are difficult to evaluate directly, and the ensiling conditions are quite varied.

Murdoch *et al.* (8), in separate tests of various amounts of chopped and unchopped forages, from 400 pounds to 13 tons, tested bisulphite additions of 0 to 16 pounds per ton. He concluded that bisulphite lowered the loss of nutrients and produced a better quality of silage. In later studies (9) they found bisulphite to be comparable to molasses in reducing dry matter losses, but that both were less effective than field-wilting the forage. Nash (10), using unreplicated treatments with uncut forages in 1400-pound capacity silos, reports that dry matter loss was reduced 5 to 7 per cent by adding bisulphite. It is generally agreed that its use lessens the objectionable odours associated with the making of grass silage.

Cordukes, Shearer and Cooper (5) showed that storage losses and quality of silage differ widely, depending on the level of dry matter and particularly on the degree of initial compaction. The objective of the present investigation was to study the effect of bisulphite on forage silages

[1]Contribution No. 115 from the Plant Research Institute and Analytical Chemistry Research Service (Contribution No. 14), Research Branch, Canada Department of Agriculture, Ottawa, Ont.

under closely controlled conditions and with sufficient replications for statistical interpretation of the results. Storage losses, nutrient preservation and acceptability by animals were used as the basis for evaluation.

MATERIALS AND METHODS

Ensiling Techniques

Tests were conducted in 1958 with 16 and in 1959 with 24 half-ton silos. Previous reports have described the type of silo used (4), the technique of harvesting the forages, of filling the silos, of examining and of sampling the resulting silages (5).

The forage mixture in the 1958 experiment was: alfalfa, 60 per cent; red clover, 5; timothy, 30; and other grasses, 5 per cent. Forages at two levels of dry matter (23 and 32 per cent) were ensiled at densities of 20 and 44 pounds per cubic foot, with and without bisulphite. Bisulphite was applied by hand at the rate of 8 pounds per ton of forage at the time of ensiling. Eight ensiling treatments were duplicated in a completely randomized design. Storage period for the experiment was 144 days.

The forage mixture in the 1959 experiment was: alfalfa, 65 per cent; timothy, 30; and other grasses and weeds, 5 per cent. Forages at three levels of dry matter (21, 28 and 36 per cent) were ensiled at an initial density of 36 pounds per cubic foot. This density was selected to provide moderate compaction in a silo and permit effective action by the bisulphite. It was necessary to wet the forage at the 21 per cent dry matter level with a garden hose after harvesting but before ensiling to provide the high moisture level for this experiment. Bisulphite was applied to the forages of each dry matter level at rates of 0, 4, 8 and 12 pounds per ton of forage, and treatments were duplicated in a completely randomized design. The silages were examined after 4 months.

Chemical Analyses

The pH values of well-mixed freshly collected material were determined. For analyses of proximate constituents duplicate sets of samples, from all the silos, were taken of the 1958 silages. One set was freeze-dried; the other was dried at 100°C. in a circulating oven, as were also all the silage samples taken in 1959. All samples were ground to pass a 20-mesh sieve in a Christy-Norris hammer mill. Official analytical procedures were followed (2), except that petroleum ether was used instead of ethyl ether in the crude fat extraction.

Palatability Tests

In 1958, the palatability of the silages was tested in four free-choice feedings with 2-year-old heifers during 2 days. Eight silages, representing the replicates of the ensiling treatments, were fed in the morning and again in the afternoon of November 13. Four heifers in each of four feeding yards were used in these tests. Two 60-pound samples of each of the eight silages were allotted at random, four to each of the four yards and then the heifers were allowed to feed free-choice for 45 minutes. After feeding, the animals were removed and what remained of each sample was weighed.

This feeding was repeated in the afternoon of the same day after each group of animals was moved to a different yard to feed on another group of silages. The animals had free access to water. This scheme of feeding was repeated on November 17 when the second replicates of the eight experimental silages were fed to the same heifers.

In 1959, the 24 silages resulting from the 12 ensiling treatments were fed free-choice to 2-year-old heifers in three feedings. In order to condition the heifers to the taste of bisulphite-treated silage, a bulked lot was placed before them the morning of October 21. That afternoon, twelve 60-pound samples, representing one replicate of the ensiling treatments, were fed in three yards, in each of which were four heifers. Each yard contained the silage samples representing one dry matter level only. The positioning of the four samples within each yard was randomized. After feeding for 45 minutes the heifers were removed and the remaining portions of the samples weighed to measure consumption. Free access to water was provided for the animals. On October 23, the second replicates of the silages

TABLE 1. — AVERAGE CHEMICAL COMPOSITION OF FRESH FORAGES BEFORE ENSILING IN 1958 (MEANS OF EIGHT SILOS)

Percentage dry matter of fresh forage	Percentage of dry matter				
	Protein	Fat	Crude fibre	Ash	Nitrogen-free extract
Low — 23.4	12.5	1.8	26.2	8.4	51.1
High — 32.2	12.9	1.9	27.0	8.1	50.1

TABLE 2. — STORAGE LOSSES OF FORAGES ENSILED AT VARYING DENSITY AND DRY MATTER LEVELS WITH AND WITHOUT BISULPHITE (MEANS OF TWO HALF-TON SILOS) 1958

Ensiling conditions			Percentage dry matter		Losses — percentage of dry matter ensiled		
$Na_2S_2O_5$ lb. per ton	Density lb. per cu. ft.	Dry matter	Fresh forage	Silage	Spoilage	Fermentation	Total
0	20	Low	23.3	13.8	26.6	48.0	74.6
0	20	High	31.8	24.7	20.6	36.2	56.8
0	44	Low	23.3	20.3	7.1	19.6	26.7
0	44	High	32.0	29.3	7.3	15.4	22.7
8	20	Low	23.4	17.0	22.7	38.0	60.7
8	20	High	31.9	23.0[1]	26.8	42.9	69.7
8	44	Low	23.6	22.9	6.4	10.7	17.1
8	44	High	33.3	28.8	8.6	19.0	27.6
Standard error of mean, 7 D.F.					1.93	3.56	4.85

[1]Single determination

were offered to the heifers in two feedings, morning and afternoon. The heifers were moved to different yards for each feeding. Thus, the 12 experimental silages were fed once to each group of heifers.

EXPERIMENTAL RESULTS

(*a*) *1958 Experiments*

The forages when ensiled were relatively uniform in chemical composition and the quality of the forage with higher dry matter was little affected by field wilting (Table 1).

Fermentation and total losses were significantly reduced as the degree of initial compaction was increased (Table 2). Because of the wide difference between storage losses at the two ensiling densities, a separate analysis was made of the data for each density. Treatment with bisulphite did not reduce ensiling losses.

There were no significant differences due to method of drying. The data shown in Table 3 are the averages from duplicate samples for both drying methods. The fat was significantly higher, the ash and pH significantly lower, in the more thoroughly compacted silages. The ash was significantly lower in the silages high in dry matter. These analyses indicated that treatment with bisulphite had no effect on high-density silages. However, on silages with low compaction, the application of bisulphite increased the protein percentage when the dry matter was low, but decreased it when the dry matter was high. Crude fibre was significantly lower when the low-density silages were treated with bisulphite.

The animals showed marked preferences for certain silages (Table 4). The highly compacted silages were significantly more acceptable. When the data were analysed on the basis of low and high density treatments, no over-all improvement in acceptability was shown after treatment with bi-

TABLE 3. — AVERAGE COMPOSITION OF SILAGES FROM FORAGES TREATED WITH BISULPHITE (MEANS OF TWO SAMPLES FROM EACH OF TWO SILOS) 1958

Ensiling conditions			pH	Percentage of dry matter				
$Na_2S_2O_5$ lb. per ton	Density lb. per cu. ft.	Dry matter		Protein	Fat	Crude fibre	Ash	Nitrogen-free extract
0	20	Low	5.5	11.5	2.2	32.6	12.9	40.8
0	20	High	5.0	14.8	2.4	31.2	10.6	41.0
0	44	Low	4.2	13.1	2.5	30.3	9.0	45.1
0	44	High	4.4	13.8	2.4	29.1	9.0	45.7
8	20	Low	5.1	13.8	2.3	27.5	12.8	43.6
8	20	High	5.8	13.5	2.4	29.9	12.2	42.0
8	44	Low	4.8	12.7	2.6	27.6	10.0	47.1
8	44	High	4.6	13.5	2.5	28.7	9.4	45.9
Standard error of mean, 8 D.F.			0.28	0.54	0.09	1.14	0.55	—

sulphite. There was a significant increase in palatability because of increased dry matter. There was a significant interaction between dry matter and the bisulphite in the high-density tests, which appears to stem from the fact that the addition of bisulphite in some way increased the palatability of the low dry matter silages.

(b) 1959 Experiments

Table 5 gives the results of analyses for pH and proximate constituents of the fresh forages in the 1959 tests. Field wilting appeared to decrease the fat and increase the crude fibre and ash content of the forage.

Statistical analyses of the storage losses (Table 6) showed no significant reductions due to bisulphite. The trend for fermentation losses to decrease with increasing rates of applied bisulphite, with silages of low and medium dry matter, was not significant. Storage losses were significantly lower in forages stored at medium to high levels of dry matter, than in those ensiled at a low level of dry matter.

TABLE 4. — FREE-CHOICE CONSUMPTION BY HEIFERS OF FORAGES ENSILED AT VARYING DENSITY AND DRY MATTER LEVELS WITH AND WITHOUT BISULPHITE 1958

Ensiling conditions			Silage acceptability — pound per heifer				
			November 13		November 18		Total
$Na_2S_2O_5$ lb. per ton	Density lb. per cu. ft.	Dry matter	a.m.	p.m.	a.m.	p.m.	
0	20	Low	1.0	1.3	0.9	0.6	3.8
0	20	High	2.7	4.0	0.4	0.3	7.4
0	44	Low	8.9	11.9	4.8	9.5	35.1
0	44	High	13.0	14.3	11.1	17.3	55.7
8	20	Low	1.0	0.8	0.1	0.3	2.2
8	20	High	0.9	0.5	0.8	0.3	2.5
8	44	Low	10.8	10.3	10.2	13.8	45.1
8	44	High	11.0	11.7	11.8	17.4	51.9
Standard error of mean, 6 D.F.							1.96

TABLE 5. — AVERAGE CHEMICAL COMPOSITION OF FRESH FORAGES BEFORE ENSILING IN 1959 (MEANS OF EIGHT SILOS)

Percentage dry matter of fresh forage	pH	Percentage of dry matter				
		Protein	Fat	Crude fibre	Ash	Nitrogen-free extract
Low — 21.4	6.0	13.6	2.4	31.9	7.6	44.5
Medium — 27.6	6.1	14.3	2.5	31.4	7.8	44.0
High — 36.9	6.1	13.7	2.1	34.9	8.6	40.7

TABLE 6. — STORAGE LOSSES OF FORAGES ENSILED AT DIFFERENT DRY MATTER LEVELS AND RATES OF APPLIED BISULPHITE (MEANS OF TWO HALF-TON SILOS) 1959

Ensiling conditions		Percentage dry matter		Losses — percentage of dry matter ensiled		
Dry matter	$Na_2S_2O_5$ lb. per ton	Fresh forage	Silage	Spoilage	Fermentation	Total
Low	0	21.4	20.2	10.9	23.4	34.3
	4	21.6	21.5	12.0	19.2	31.2
	8	21.2	21.6	14.7	17.7	32.4
	12	21.2	22.1	12.0	15.1	27.1
Medium	0	27.6	25.7	10.1	17.9	28.0
	4	28.0	26.9	8.3	14.1	22.4
	8	27.6	26.3	9.8	16.0	25.8
	12	27.2	26.0	13.6[1]	14.9[1]	28.5[1]
High	0	37.0	33.6	11.5	16.6	28.1
	4	36.6	33.0	10.7	17.4	28.1
	8	37.5	33.9	10.5	16.9	27.4
	12	36.5	34.3	11.7	14.5	26.2
Standard error of mean, 10 D.F.				1.25	2.56	2.52

[1]Calculated partially on basis of missing value technique

TABLE 7. — AVERAGE COMPOSITION OF SILAGES FROM FORAGES TREATED WITH BISULPHITE (MEANS OF TWO SILOS) 1959

Ensiling conditions		pH	Percentage of dry matter				
Dry matter	$Na_2S_2O_5$ lb. per ton		Protein	Fat	Crude fibre	Ash	Nitrogen-free extract
Low	0	4.9	12.1	3.3	37.2	8.0	39.4
	4	4.7	13.9	3.2	33.8	8.2	40.9
	8	4.9	14.4	2.9	33.1	8.6	41.0
	12	5.3	14.3	3.0	32.9	9.2	40.6
Medium	0	4.6	15.0	3.0	32.8	8.3	40.9
	4	4.7	14.5	2.8	33.9	8.0	40.8
	8	5.0	14.6	2.8	32.6	9.1	40.9
	12	4.8	14.6	2.8	32.5	9.0	41.1
High	0	5.2	12.9	3.1	35.6	8.3	40.1
	4	5.1	13.4	3.0	34.8	8.6	40.2
	8	5.2	12.9	2.9	36.3	8.5	39.4
	12	5.2	13.2	2.8	33.6	9.4	41.0
Standard error of mean, 12 D.F.		0.15	0.17	0.17	0.58	0.29	—

TABLE 8. — FREE-CHOICE CONSUMPTION BY HEIFERS OF FORAGES ENSILED AT DIFFERENT DRY MATTER LEVELS AND RATES OF APPLIED BISULPHITE 1959

Ensiling conditions		Silage acceptability — pound consumed per heifer			
Dry matter	$Na_2S_2O_5$ lb. per ton	October 21 p.m.	October 23		Total
			a.m.	p.m.	
Low	0	3.1	2.1	3.3	8.5
	4	9.4	7.2	5.7	22.3
	8	3.9	6.8	10.3	21.0
	12	5.1	6.7	4.5	16.3
Medium	0	6.2	3.2	2.9	12.3
	4	5.7	6.4	6.8	18.9
	8	7.9	9.5	8.4	25.8
	12	2.4	0.0	3.2	5.6
High	0	5.0	5.5	5.8	16.3
	4	2.5	2.5	3.4	8.4
	8	6.5	0.5	3.4	10.4
	12	1.7	1.8	1.9	5.4
Standard error of mean, 18 D.F.					1.02

The pH and percentages of the proximate constituents in the dry matter of the various silages are given in Table 7. The dry matter of the silages affected significantly the pH, as well as protein and crude fibre contents. The best preservation of protein and the lowest pH values and crude fibre percentages for silages were at the medium level of dry matter. Treatment with bisulphite significantly increased the protein of low dry matter silages. In these low dry matter silages, the crude fibre decreased and the ash increased with increasing rates of applied bisulphite.

There were differences in the acceptability of the silages by the heifers (Table 8). Statistical analyses showed that the dry matter and the use of bisulphite significantly influenced the acceptability of the various silages. With the low dry matter silages, all rates (4, 8 and 12 pounds per ton) of bisulphite increased acceptability as compared with the 0-pound rate. The 12-pounds-per-ton application had the least effect of the three rates but consumption was still twice that of the check treatment. Within the medium dry matter silages the 4- and 8-pounds-per-ton rates increased acceptability whereas the 12-pound rate markedly decreased it. All rates of bisulphite applied to forages of high dry matter decreased the palatability of the resulting silages.

DISCUSSION AND CONCLUSIONS

Treatment with bisulphite did not reduce storage losses significantly in any of the experiments. Compaction and dry matter had a decided effect on storage losses. Initial compaction and the level of dry matter in the crop at the time of ensiling had more effect on losses than the use of bi-

sulphite. Spoilage losses appear to be high. Since a perforated cover of wood was used and no attempt was made to seal out the air the ratio of spoilage losses would be higher than in a standard upright silo.

Silage quality and odour were improved by use of bisulphite particularly with the low dry matter forages. Protein was preserved better when bisulphite was applied to forages of low dry matter which received low or inadequate compaction. Treated silages in general had lower crude fibre, which indicates better preservation.

The acceptance of the silages by heifers was affected by treatment with bisulphite. Their acceptability was improved when forages of low to medium dry matter were ensiled with moderate compaction. The acceptability was not affected of treated forages of low dry matter which received poor compaction or of high dry matter forages ensiled with thorough compaction.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to P. Robinson and the Statistical Research Service for advice and assistance with the statistical analyses, to the Animal Research Institute for design of, and assistance with acceptability trials, and to J. H. A. Labelle and G. Morris for the chemical analyses.

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THE VEGETATIVE PROPAGATION OF *AMELANCHIER ALNIFOLIA*

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[Received for publication February 23, 1961]

ABSTRACT

A 3-year study of methods of vegetatively propagating *Amelanchier alnifolia* has shown that: 1. hardwood cuttings are difficult to root; 2. root cuttings root readily; 3. leafbud cuttings show some promise but require further study; 4. softwood cuttings root readily if they are taken when the new growth is 3 to 6 inches long and placed in a mist propagating frame. Bottom heat at 70°F. lengthens the time when softwood cuttings can be taken while 0.3 per cent IBA in talc increases rooting.

INTRODUCTION

Amelanchier alnifolia is a hardy native shrub widely distributed in the Canadian prairies and parts of the United States. It not only makes an attractive ornamental but the fruit is used for desserts, particularly in areas where the commonly cultivated fruits are not completely hardy. *Amelanchier* belongs to the family Rosaceae, sub-family *Pomoidaea*. According to Bailey (1) *Amelanchier* can be propagated from seed or root sprouts, while Osborne (4) states that they can also be propagated from cuttings. These methods were not satisfactory with seedling selections at Beaverlodge because seed development was not completely apomictic, sucker development was variable, and hardwood cuttings would not root.

A satisfactory method of propagating *A. alnifolia* was needed, so that clonal stocks of the hybrids and selections developed at Beaverlodge could be distributed. In 1958 the objectives were to determine: 1) the effect of mist propagation on rooting; 2) the relative ease of rooting hardwood, softwood, root and leafbud cuttings, and 3) the best time to take cuttings. In 1959 and 1960 the objectives were to determine, using the most promising type of cutting, whether; 1) bottom heat increased rooting, and 2) rooting compounds promoted rooting or extended the period when the cuttings would root.

MATERIALS AND METHODS

In 1958, hardwood cuttings were taken at monthly intervals from mid-October to mid-April. Cuttings taken before February were stored at 32°F. in sealed polyethylene bags until they were placed in a bottom-heated propagating bed in a cool greenhouse. Cuttings taken in February and later were put directly into the bed. Leafbud and softwood cuttings were taken every 2 weeks from the end of May until the middle of August. Root cuttings, approximately 10 millimetres in diameter, were taken every 2 weeks from the end of May until the end of July.

Each lot of cuttings was divided into six groups of ten each. Three groups were placed in a glass-covered cold frame and watered to maintain humidity, and three placed in an uncovered polyethylene-sided mist propagating frame. Both frames were heated with buried electric cables.

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In both 1959 and 1960, softwood cuttings were taken at approximately weekly intervals for 3 or 4 weeks, commencing when the young growth was 3 to 4 inches long. Four rooting compounds and a control were used, namely: 0.1, 0.3, and 0.8 per cent indolbutyric acid (IBA) in talc[1], a chlorophyll plant extract (CH)[2], and the control. Duplicate blocks, one with bottom heat and one without, were laid out in a mist propagating bed in a split-plot design with dates of planting forming the main treatments and the rooting compounds the sub-treatments.

In all years cuttings were taken from seedlings of *A. alnifolia* var. Smoky, the rooting mixture consisted of two parts sand and one part peat, and bottom heat was controlled at 68 to 70°F. In 1959 the cuttings were taken from young root sprouts and mature branches, whereas in 1960 cuttings were taken only from young root sprouts.

Analysis of each block was based on the method outlined by Cochrane and Cox (2) for split-plot experiments, while comparisons between means were estimated at the 5 per cent level of significance by Duncan's multiple range test as discussed by Robinson (5). Differences between heated and unheated beds was measured by the "t" test (3).

RESULTS

Hardwood cuttings were not satisfactory for propagating *Amelanchier* as only 1 per cent calloused and none rooted.

Root cuttings grew readily, the average yield for all dates being 90.4 per cent without mist and 91.6 per cent with mist. Softwood and leafbud cuttings failed to root without mist. Under mist, 13.3 per cent of the leafbud cuttings rooted when taken June 10 and 26, but failed to root when taken at later dates. Under mist, roots developed on 40.0 per cent of the softwood cuttings taken on May 27 and 46.7 per cent of those taken June 11. Yield of rooted cuttings dropped to 13.3 per cent for the June 26th collection date and to zero for later dates.

Although softwood cuttings required mist and yielded less than root cuttings they were used in subsequent experiments because they were readily obtained and because it was hoped that improved techniques would improve their rooting.

Optimum yields of rooted softwood cuttings were obtained on June 15 in 1959 and June 10 in 1960 in both the heated and unheated beds (Table 1). At this time the new growth had reached a length of 3 to 6 inches. Bottom heat did not significantly increase the yield of rooted softwood cuttings nor alter the optimum time for taking cuttings. However, the bottom heat, particularly in 1960, tended to extend the period when cuttings could be rooted.

Differences between cutting dates in 1960 were not significant in the heated beds, but in the unheated beds the yield from the June 28th cuttings was significantly lower than from both the previous dates. Similar trends were obtained in 1959 with the yield from June 29th cuttings in the heated

[1]Hormodin 1, Hormodin 2 and Hormodin 3, respectively (Merck & Co. Ltd., Montreal, Que.)
[2]Chloromone (Chlorophyll Corp.)

beds proving significantly lower than from the June 8th and 15th cuttings, and in the unheated beds significantly lower than from the June 15th and 22nd cuttings.

In 1959, in the heated bed, cuttings treated with 0.3 and 0.8 per cent IBA produced significantly greater numbers of rooted plants than the check,

TABLE 1. — PERCENTAGE YIELD OF ROOTED SOFTWOOD CUTTINGS FROM ALL TREATMENTS

1959	June			
	15	8	22	29
Bottom heat at 68-70°F.	[1]74.7	50.7	42.7	12.0
	June			
	15	22	8	29
Without bottom heat	64.6	42.0	37.3	9.6

1960	June		
	10	22	28
Bottom heat at 68-70°F.	84.7	68.7	66.0
Without bottom heat	87.3	69.3	42.7

[1]Differences between underlined figures are not significant at P = .05.

TABLE 2. — PERCENTAGE YIELD OF ROOTED SOFTWOOD CUTTINGS OBTAINED AT ALL DATES

	Per cent IBA			Chloro-mone	Check
	0.8	0.3	0.1		
1959					
Bottom heat at 68-70°F.	[1]57.7	57.7	45.8	43.3	20.8
Without bottom heat	65.0	38.3	37.5	34.2	16.7
1960					
Bottom heat at 68-70°F.	67.8	86.7	75.6	65.6	70.0
Without bottom heat	70.0	78.9	64.4	63.3	55.6

[1]Differences between underlined figures are not significant at P = .05.

whereas only the 0.8 per cent treatment did so in the unheated bed (Table 2.) In 1960, the 0.3 per cent IBA treatment produced the largest number of rooted cuttings in both the heated and unheated beds but there were no significant differences between any treatments. The differences between means for both years and the interaction between dates and rooting compounds were not significant.

DISCUSSION

It has previously been reported (4) that *Amelanchier* can be propagated from cuttings but the type of cutting was not specified. In the work reported here it has been shown that hardwood cuttings of *A. alnifolia* are difficult, if not impossible, to root, but that leaf bud, root and softwood cuttings can be rooted.

Although a maximum of 13.3 per cent of the leaf bud cuttings rooted the yield could probably be increased with improved techniques. Root cuttings shoot readily and appear to be the only type of cutting which can be grown without mist. However, despite the high yield of plants from root cuttings, they are impractical because of the difficulty of obtaining material without damaging the plant.

Propagation from softwood cuttings, on the other hand, is quite practical. They are readily obtained, can be removed without danger of killing the parent plant and, if the proper techniques are used, good yields of rooted cuttings result. Good rooting of softwood cuttings is mainly dependent on taking the cuttings at the correct stage of development and maintaining them in a moist atmosphere until they root. Bottom heat and rooting compounds are not essential but both tended to increase the yield; bottom heat by lengthening the period when softwood cuttings will root, and rooting compounds, particularly 0.3 per cent IBA, by increasing the percentage of cuttings that root at all dates.

A low precipitation of 1.7 inches in June 1959 resulted in early maturity of the new growth while a precipitation of 6.05 inches in 1960 caused new growth to remain soft for a longer period. Despite the differences in the condition of the cuttings, results were similar in both years. The only difference between years was in the unheated beds where the 0.8 per cent IBA treatment produced the greatest number of rooted cuttings in 1959 but in 1960 produced less than the 0.3 per cent IBA treatment.

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PLANT CHROMOGENS AS AN INDICATOR IN ESTIMATING THE DIGESTIBILITY OF FORAGE CROPS BY SHEEP

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[Received for publication February 23, 1961]

ABSTRACT

Digestibilities of 28 forages were determined in conventional digestibility trials with sheep. The concentration of chromogens was measured as the absorptivity at 406 mμ in extracts of the forages and resultant feces. The concentration of extracted chromogens was found to be affected by light, temperature, and time.

The regressions of the digestibility of the forages on the concentration of chromogens in both the forages and the resultant feces were significant at $P < 0.001$. However, the data varied too much for a reliable estimation of the digestibility from the concentration of chromogens in either forages or feces. The recovery of chromogens from the feces was unsatisfactory for determining the digestibility by the chromogen-ratio method in 26 of the forages studied.

An examination of the visible spectral absorbance curves of the chromogens suggested a consistent absorption peak for the forage extracts at 413 mμ. In each case the resultant feces curve crossed the forage curve on both its slopes. These intercepts are suggested to account for the two isosbestic points (406 and 415 mμ) proposed by other workers. However, in this study the intercepts varied too much to suggest that either of them would be reliable for measuring the concentration of chromogens for digestibility determinations.

INTRODUCTION

Reid *et al.* (7) introduced the theory that plant pigments (chromogens) could be used as an internal indicator to estimate the digestibility and intake of forage by sheep and cattle. They measured the concentration of chromogens in extracts of forages and feces as the spectrophotometric absorbance (A)[2] at 406 mμ; this wavelength was reported to be consistently near an isosbestic point for chromogens in corresponding forages and feces. In order to avoid extraction of chromogens from forages, Reid *et al.* (8) expressed the relationship between the digestibility of the forages and the concentration of chromogens in the resultant feces by a regression equation. From the significance of this regression it was suggested that the digestibility of a forage could be estimated from the concentration of chromogens in its resultant feces.

The work reported herein was conducted to test the reliability of the use of chromogens as an internal indicator when determining the digestibility of native and cultivated forage crops grown in the semi-arid region of the Canadian Prairies. In the course of developing the laboratory procedure, a limited study was carried out on the effects of time, temperature, light intensity, and type of spectrophotometer on the apparent concentration of chromogens in extracts of forages and feces.

MATERIALS AND METHODS

Twenty-eight forages were fed to wether lambs in conventional digestibility trials. Twenty-five of these were harvested as hay and fed during the subsequent fall and winter, while three were harvested daily and fed

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[2]Spectrophotometric nomenclature used is that proposed by A.O.A.C. Committee on Spectrophotometry. J. Offic. Agr. Chemists 37:54. 1954.

fresh during the early summer. The experimental designs included five Latin squares and a balanced incomplete block (10) with three to ten replications. Although these trials were not carried out simultaneously or with the same group of sheep the conditions were sufficiently standardized to warrant the assumption that the data from the various tests were comparable. Yearling wether lambs of Rambouillet breeding were used in all experiments. In each case they were confined in digestion stalls designed for separate collection of feces and urine. During pre-collection periods they were exercised in a small corral each afternoon. Each feeding period continued for 21 days, and comprised a pre-collection period of 11 days followed by a collection period of 10 days. During each collection period the total feces from each sheep was collected daily and frozen.

The forages fed as hay were all chopped into 1- to 1½-inch lengths by a forage cutter prior to the start of a trial. The forages fed green were harvested every second day and chopped in a similar manner as the hays. During pre-collection periods each lamb's appetite for a particular feed was determined by increasing or decreasing its ration according to the amount refused. Half of each day's ration was fed at 9.00 a.m. and half at 4.00 p.m. At the end of the pre-collection periods the pre-determined amounts of the rations were weighed into paper bags for each feeding during the collection period. Samples were removed for analyses when the rations were being parcelled for the collection periods. The forages fed green were weighed out and sampled as soon as possible after harvesting. Dry matter determinations were carried out at once and samples for chromogen determinations were frozen until the time of the analyses. Refused portions of any feed were removed from the feeders and returned to their respective bags prior to each feeding; at the end of the collection period these were bulked and prepared for analyses. Whenever the chromogen content in refused feed differed markedly from that in the total feed, the concentration of chromogen in the latter was adjusted in proportion to the difference and according to the amount of refused feed.

The hay samples were ground through a 40-mesh screen in a laboratory hammer mill; sub-samples were removed for dry matter analysis at the time of the chromogen extraction. The frozen forage samples were extracted as they were and sub-sampled for dry matter analysis. The total feces collected from each animal in each period was mixed in a batch mixer. The moisture content was increased to 87 or 88 per cent by adding water to give a semi-liquid consistency. A sample of this was homogenized in a Waring blender, poured into ¾-oz. paper portion cups and re-frozen in this form. When the chromogens were extracted, a second sample was analysed simultaneously for dry matter.

Extraction of chromogens was carried out as recommended by Reid *et al.* (7). A Waring blender and a steel blender cup with a tight-fitting screw lid were used. A surface cooler mounted in the lid controlled the temperature of the solvent during the extraction period. Each sample was macerated for two periods of 10 minutes in 200 millilitres of solvent. The concentration of chromogens in the extracts was measured in a Beckman DU

spectrophotometer at 406 mμ as recommended by Reid *et al.* (7); for convenience it was expressed in "milli-absorptivity" (a). A stipulation to this term is that it was calculated on the basis of the total weight of dry matter extracted rather than the traditional weight of dry matter dissolved.

Fresh samples of forages and feces were extracted to observe the effect of time, temperature and light on the absorptivity of chromogens. The absorbance (A) of the extract was obtained immediately after the extraction. The extract was then divided into six lots which were randomly distributed to the following treatments in a 3 by 2 factorial design. Two lots were protected against light, two lots were exposed to electric light equal to ordinary laboratory conditions (125 to 150 lumens) for 2 hours, and two lots were exposed to direct sunlight (8000 to 9000 lumens) for 2 hours. One lot from each of the "light" treatments was then stored in a refrigerator at 0 to 5°C., while the other lot was stored at room temperature at 18 to 24°C. The absorbances of all six treatments were obtained at 2, 5, 20, 44, 68, and 360 hours after extraction.

RESULTS AND DISCUSSION

Evidence of the influence of light, temperature, and time on A of chromogen extracts is summarized in Table 1. In extracts protected against light, A increased approximately 33 per cent during 360 hours. Refrigeration slowed down the rate of increase in the earlier part of this period. Thus, after 2 hours A had increased 0.9 per cent in the refrigerated extract as compared to 3.7 per cent at room temperature; after 5 hours the increases were 1.9 and 6.5 per cent respectively. At 360 hours no difference was apparent. Two hours of electric light caused an immediate increase in A of 17 per cent while two hours of sunlight caused an immediate drop of 42 per cent. The changes due to light exposure eliminated the effect of storage temperature. These observations correspond to the results by Lancaster and Bartrum (5) and indicate the need for refrigeration, protection against light, and measurement of A as soon as possible after extraction.

TABLE 1. — THE EFFECT OF STORAGE TIME, STORAGE TEMPERATURE AND LIGHT EXPOSURE UPON THE ABSORBANCE (A) OF CHROMOGEN EXTRACTS AT 406 mμ

Hours after extraction	No light		Electric light		Sunlight	
	0-5°C.	18-24°C.	0-5°C.	18-24°C.	0-5°C.	18-24°C.
	A	A	A	A	A	A
0	.535	.535	.535	.535	.535	.535
2	.540	.555	.625	.625	.310	.310
5	.545	.570	.625	.630	.312	.310
20	.585	.625	.625	.628	.310	.311
44	.615	.665	.627	.627	.309	.312
68	.640	.685	.630	.628	.310	.310
360	.705	.710	.631	.629	.308	.309

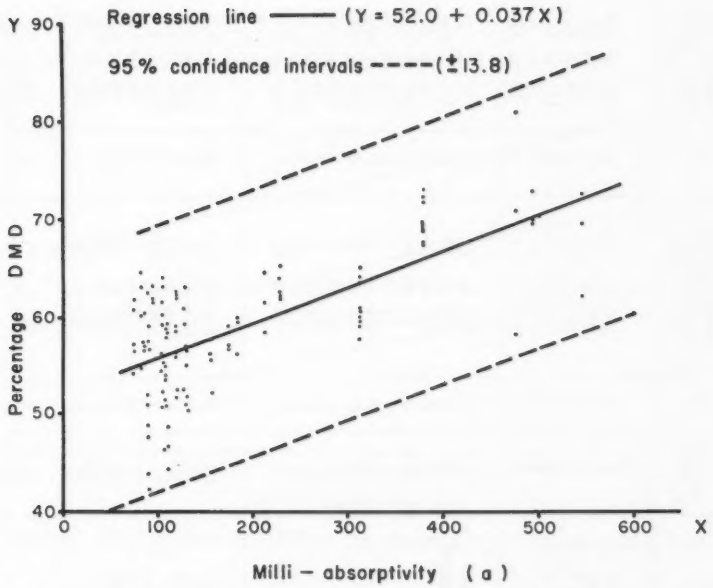

FIGURE 1. The regression of DMD on the concentration of chromogens in forages.

A mixture of chromogen extracts of forages and feces did not conform to Beer's Law when measured at 406 mμ in a Coleman 6A spectrophotometer, whereas the conformity was excellent on the Beckman DU. This discrepancy was considered due to a difference in band width between the two instruments as suggested by Goldenberg (3). The function of A (Y) on the relative concentration of chromogens (X) in the extracts for the two instruments was:

$$\text{Beckman DU: } Y = 0.128X \tag{1}$$

$$\text{Coleman 6A: } \log Y = 0.0024 + 0.009 \log X \tag{2}$$

These equations were found to be useful in converting results obtained by one instrument to a basis comparable to those obtained by the other.

The relationship between (a) and the dry matter digestibility (DMD) (Figure 1) was determined because the colour of a forage generally is a good indicator of its maturity and/or the success with which it has been conserved. The regression of DMD (Y) on the milli-absorptivity (X):

$$Y = 52.0 + 0.037X; (\pm 13.8) \tag{3}$$

was significant at $P < 0.001$ with 113 degrees of freedom (DF). Despite this high degree of significance the 95 per cent confidence interval of Y for any value of X is ±13.8 per cent. This means that the probability is 95 per cent that the actual value of a DMD lies in these intervals. The

TABLE 2. — THE CONCENTRATION OF CHROMOGENS IN 28 FORAGES AND THEIR PER CENT RECOVERY FROM SHEEP FECES

Description of forages		Forage number	N	Concentration of chromogen (milli-absorptivity)	N	Recovery of chromogen (per cent)
Native hays						
Rough fescue, *Festuca scabrella* Torr.	Harvested in Cypress Hills 1953	1	4	130 ± 11*	4	100 ± 21*
	Harvested in Cypress Hills 1954	2	4	108 ± 11	4	95 ± 17
	Harvested in Alberta Foothills 1953	3	4	107 ± 11	4	81 ± 12
	Harvested in Alberta Foothills 1954	4	4	132 ± 9	4	114 ± 11
Western wheatgrass, *Agropyron smithii* Rydb.	Harvested in Walsh Flat 1953	5	4	119 ± 12	4	98 ± 8
	Harvested in Walsh Flat 1954	6	4	90 ± 3	4	85 ± 17
Cultivated hays						
Reed Canary grass, *Phalaris arundinacea* L.	Immature aftermath, cut late fall	7	6	313 ± 15	10	107 ± 7
	Early flowering, 4 feet tall, stemmy	8	4	171 ± 15	3	144 ± 76
Crested wheatgrass, *Agropyron cristatum* (L.) Gaertn.	Late bloom stage	9	4	82 ± 20	4	94 ± 17
	Dough stage, leafy	10	4	85 ± 23	4	102 ± 10
Intermediate wheatgrass, *A. intermedium* (Host.) Beauv.	Early seed stage	11	5	74 ± 4	5	101 ± 11
	Early seed stage, leafy	12	5	103 ± 7	5	119 ± 22
Streambank wheatgrass, *A. riparium* Scribn. and Smith	Culm formation very sparse	13	5	88 ± 5	4	101 ± 20
	Culm formation sparse	14	5	109 ± 5	3	101 ± 21
Russian wild ryegrass, *Elymus junceus* Fisch.	Basal leaves only	15	5	94 ± 4	3	81 ± 9
	Culm formation very sparse	16	5	117 ± 20	4	105 ± 7
Tall wheatgrass, *A. elongatum* (Host.) B.P.	Early flowering stage, 4-5 feet tall	17	5	108 ± 12	4	150 ± 37
Mixed alfalfa-grass hays						
Ladak alfalfa, *Medicago media* Pers.	Early flowering stage	18	3	212 ± 117	3	126 ± 74
	Medium flowering stage	19	5	227 ± 76	5	213 ± 25
Ladak alfalfa and rough fescue	Mixture 2:1	20	3	183 ± 65	3	109 ± 24
	Mixture 1:2	21	3	156 ± 52	3	107 ± 25
Ladak alfalfa and crested wheatgrass	Mixture about 1:2, flowering	22	3	127 ± 9	4	94 ± 13
	Mixture about 2:1, pre-flowering	23	7	379 ± 35	10	98 ± 12
Ladak alfalfa and oat hay	Mixture 1:4	24	4	104 ± 29	5	92 ± 18
	Mixture 1:4, dried at 100°C.	25	5	89 ± 24	4	100 ± 5
Harvested daily and fed fresh						
Ladak alfalfa and timothy, *Phleum pratense* L.	Mixture about 1:4, immature	26	4	493 ± 11	5	89 ± 19
Ladak alfalfa and intermediate wheatgrass	Mixture about 2:3, immature	27	4	476 ± 113	3	85 ± 24
Ladak alfalfa and Russian wild ryegrass	Mixture about 3:2, immature	28	4	546 ± 66	4	80 ± 20

N = Number of observations *Confidence Intervals $P < 0.05$ (9)

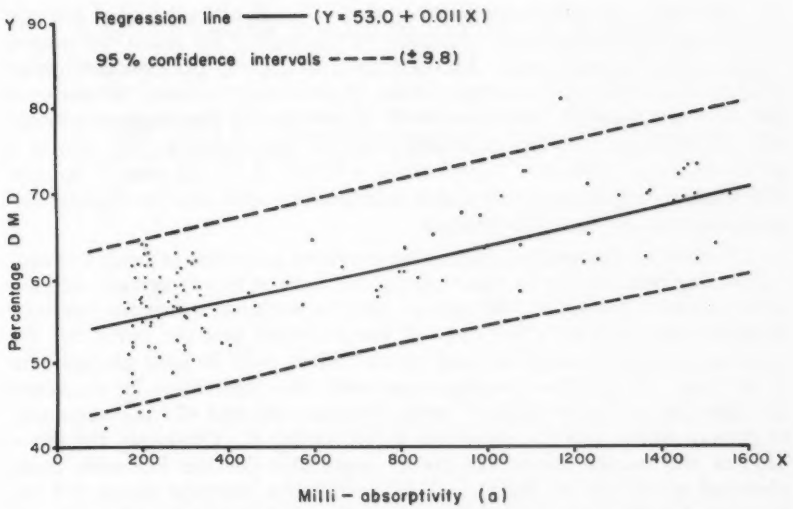

FIGURE 2. The regression of DMD on the concentration of chromogens in feces.

consequence of this is that the DMD cannot be estimated with a satisfactory accuracy from the concentration of chromogens in the forages unless other major variables can be eliminated from the observations. The plot of Y on X (Figure 1) did not reveal the presence of any single major variable, indicating that the number of variables was too great for any one to be sufficiently represented to show up in the observations made. For this reason no attempts were made to measure the significance of deviation from linearity of Y on X in any of the four groupings of forages listed in Table 2. The magnitude of sampling error within the forages is indicated by the confidence intervals on (a) in column 5.

As a check on the proposal by Reid *et al.* (8) that the digestibility of a forage could be predicted from the concentration of chromogens in its resultant feces, the regression of the DMD (Y) on the milli-absorptivity of chromogens in the feces (X) was calculated (Figure 2) for the forages listed in Table 2. This regression:

$$Y = 53.0 + 0.011X; (\pm 9.8) \qquad (4)$$

was also significant at $P < 0.001$ with 113 degrees of freedom. Despite this high level of significance, the 95 per cent confidence intervals of Y for any value of X was far too great to allow a prediction of the DMD from the value of (a) with a satisfactory degree of accuracy. The plot of the DMD on (a) (Figure 2) did not reveal the presence of any additional major variable which could be eliminated by a non-linear regression or by dividing the data into various groups for separate regressions. This again suggested that too many variables were involved to allow any one to be recognized in the sample of forages studied.

The recovery of chromogens from the feces of each sheep fed the forages listed in Table 2 was calculated on the basis of the mean chromogen concentration in the forage. The data show that the 95 per cent confidence interval was restricted to a range within 10 percentage units of 100 per cent for only two forages. As an error of 10 per cent in chromogen recovery will cause an error in the estimated DMD ranging from 10 per cent at a DMD of 50 per cent to 2.5 per cent at a DMD of 80 per cent, it appears that the chromogens were unreliable as an internal indicator for digestibility determinations by the ratio method.

A study of the spectral absorbance curves of a number of feeds and corresponding feces similar to that reported by Reid *et al.* (7) agreed with the observation by Brisson and Hatina (1) that the forage chromogens exhibited an absorption peak near 413 mμ. It was observed that the curve for the feces in each case crossed the feed curve "cutting off" its peak on each side of 413 mμ. These two crossings agree with the observation by Davidson (2) that the isosbestic point(s) varied between 390 and 425 mμ according to type of forage and the individual animal eating it. Obviously the intercept of the curves below 413 mμ is responsible for the isosbestic point observed at 406 mμ by Reid *et al.* (7), while the intercept above 413 mμ accounts for the point at 415-416 mμ, indicated by other workers (2, 4). It was also observed that the absorption peak at 413 mμ was rather steep and that 406 and 415-416 mμ were on the slopes of the peak. This could explain much of the variability in the recovery of chromogens since measurements on steep slopes are likely to cause larger errors. This agrees with the observations of Raymond *et al.* (6). Various other features of the spectral absorbance curves in the visible range indicated interesting clues that might prove fruitful in further studies in the use of plant pigments as an internal indicator in digestibility determinations.

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GERMINATION OF SWEET CHERRY (*PRUNUS AVIUM* L.) POLLEN *IN VITRO* AS INFLUENCED BY FUNGICIDES

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[Received for publication May 18, 1961]

ABSTRACT

Effects of fungicidal concentrations of sulphur, dichlone, ferbam, and captan on sweet cherry (*Prunus avium* L.) pollen germination and growth were studied in artificial media. Sulphur did not reduce the germination of pollen. Dichlone and ferbam reduced germination from 53.0 per cent to 47.1 per cent and 40 per cent, respectively, reductions which were significant statistically. Sprayed at 0.2 pound/100 gallons or less, captan did not reduce germination. Captan, sprayed at 2 pounds/100 gallons, almost entirely prevented pollen germination, and arrested the elongation of pollen tubes.

INTRODUCTION

Rich (2) found that apple pollen would not germinate in sucrose solutions containing the fungicides captan, glyodin, dichlone, or ferbam. Sulphur apparently had little effect. He concluded, however, that the *in vitro* methods he employed were too severe in their effects on pollen germination and that none of the fungicides used reduced pollen germination or fruit-set when applied to apple trees in bloom.

For the control of sweet cherry blossom blight, *Sclerotinia fructicola* (Wint.) Rehm, the recommended fungicides are wettable or paste sulphur, ferbam, captan, or dichlone (1). It is recommended that the spray be applied just before blossoms open and that, if weather is wet, the application be repeated. Therefore, it is entirely possible that under some conditions one of these fungicides might be applied to open flowers of the crop concerned. Nothing was known of the effect of these sprays on stone-fruit pollen germination, or the growth of pollen tubes. This paper reports an investigation of these questions. Since sweet cherry fruit-set is more uncertain than that of other stone fruits, the present experiment was devoted to effects on sweet cherry pollen.

MATERIALS AND METHODS

Several fungicides were used at the concentrations recommended for the control of blossom blight. The preparations used were 6 pounds/100 gallons pre-pick wettable sulphur (99 per cent sulphur); 2 pounds/100 gallons 50 per cent captan (N-trichloromethylmercapto-4-cyclohexene-1, 2-dicarboximide); ½-pound/gallons 50 per cent dichlone (2,3-dichloro-1, 4-naphthoquinone); and 2 pounds/100 gallons ferbam (76 per cent ferric dimethyl-dithiocarbamate).

The medium for pollen germination contained 10 per cent sucrose and 0.75 per cent agar. Boron at the rate of 3 p.p.m. was added as boric acid. [The concentration of boron added was based upon the results of Thompson and Batjer (3) and upon the fact that the distilled water used in this experiment was drawn from Pyrex glass pipes and therefore probably contained some boron already.] The medium was flowed on to glass slides and pollen was dusted finely over the medium. The slide was placed in a petri dish, containing wet filter paper, as a moist chamber. The fungicides were

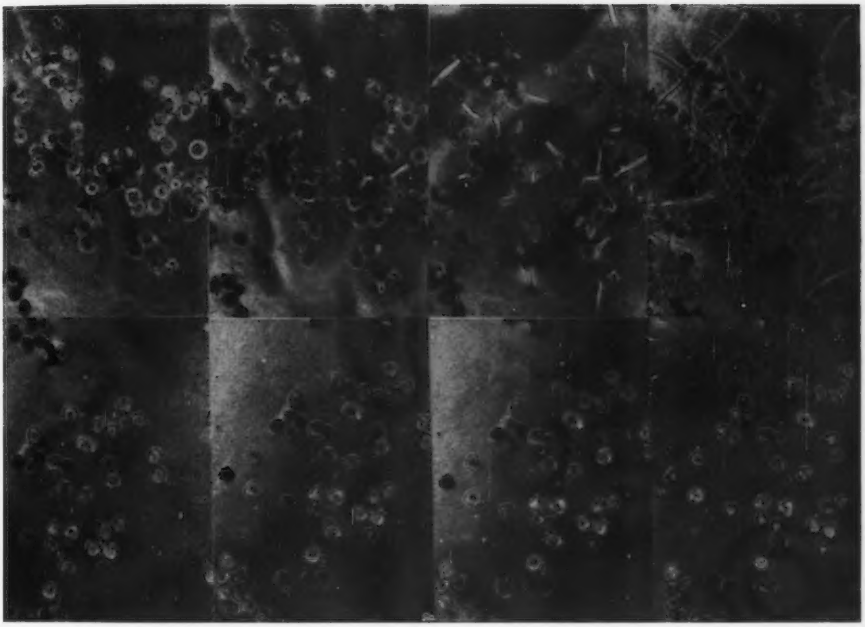

FIGURE 1. Sweet cherry pollen sprayed with 2 pounds/100 gallons captan (*bottom*) and check. *Left to right*, after 30, 50, 70, and 990 minutes of incubation. (55X.)

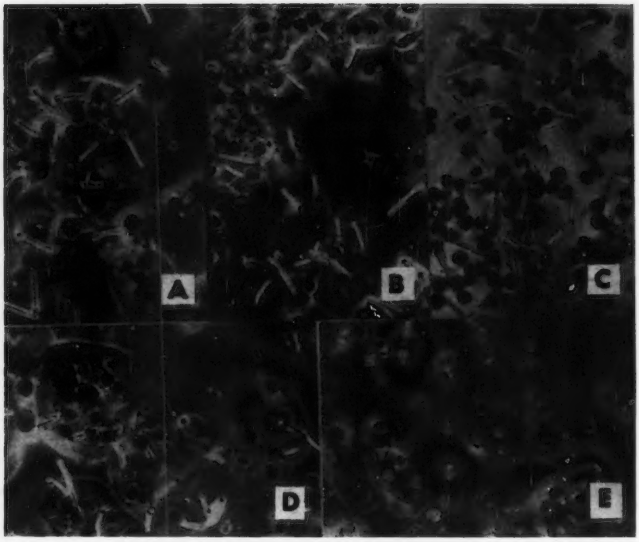

FIGURE 2. Sweet cherry pollen sprayed with fungicides in the concentrations recommended for orchard application. Photographed after 75 minutes' incubation — A) check, B) sulphur, C) dichlone, D) ferbam, E) captan. (55X.)

sprayed in a fine mist from an atomizer at about 15 pounds pressure. The slides were exposed to the spray for 5 seconds so that the fungicide was applied without "run-off" in a thin uniform mist over the entire pollen area. The slides were then incubated in their individual moist chambers for 75 minutes except where otherwise noted. Commercial cherry pollen* collected in 1960 was used.

In most of the counting, the slide was moved across the field of view beginning at one side. Every pollen grain and every clearly recognizable pollen tube passing the field of view was counted. The results were recorded at the end of each 100 counts until a total of at least 500 pollen grains had been counted. Each replicate included one slide subjected to each of the treatments.

Preliminary experiments had shown that germination of untreated pollen was complete after incubation for 75 minutes on agar at 25°C. (Figure 1). This time was used as a standard incubation time except where otherwise noted.

All photographs were taken on 35-mm. film with a Zeiss photomicroscope using phase contrast optical equipment.

RESULTS

Sulphur, Dichlone, Ferbam, Captan; and Sweet Cherry Pollen Germination

The fungicides sulphur, dichlone, ferbam, and captan were immediately sprayed on the pollen on agar in concentrations used in orchard application. Photographs and pollen germination counts were made immediately after 75 minutes of incubation. The counts are summarized in Table 1. The checks in replicates 1 and 2 were untreated; the checks in replicates 3, 4, and 5 were sprayed with tap-water. The germination of pollen in the captan treatments in replicates 1 and 5 respectively were 12.2 per cent and 4.2 per cent (Table 1). The pollen grains that contributed such high counts in the captan treatments were found germinating normally in little "islands" that had apparently escaped contact with the fungicide. All of the remaining pollen in those slides was apparently killed.

*The cherry pollen was obtained from L. C. Antles Pollen Supply & Associates Inc., Wenatchee, Wash.

TABLE 1. — EFFECT OF FUNGICIDES ON SWEET CHERRY POLLEN GERMINATION

Treatments[1]	Replicates					Average[2]
	1	2	3	4	5	
Check[3]	60.8	55.0	54.4	49.6	45.1	53.0
Sulphur	55.7	47.6	48.0	54.4	55.6	52.3
Dichlone	48.8	51.8	41.4	45.0	48.7	47.1
Ferbam	45.0	35.4	37.2	45.3	37.2	40.0
Captan	12.2	0.0	0.0	0.0	4.2	3.3

[1]The fungicides were all sprayed in the concentrations recommended for the control of blossom blight in the orchard.
[2]L.S.D. 5% 5.68
[3]Checks were untreated in replicates 1 and 2, sprayed with tap-water in replicates 3, 4, and 5.

TABLE 2. — EFFECT OF CAPTAN CONCENTRATION ON SWEET CHERRY POLLEN GERMINATION

Treatments	Replicates					Average[1]
	1	2	3	4	5	
Check[2]	55.5	53.2	47.0	43.6	17.4	43.3
0.002 lb./100 gal.	51.8	49.3	53.0	31.0	47.2	46.5
0.02 lb./100 gal.	41.8	51.8	42.1	47.2	45.2	45.6
0.2 lb./100 gal.	56.3	39.6	41.6	44.8	46.0	45.7
2 lb./100 gal.	8.2	6.4	3.2	3.2	2.8	4.8

[1]L.S.D. 5% 9.5
[2]The checks were sprayed with tap-water.

From the data in Table 1, using the 5 per cent level of significance, sulphur and dichlone were not different from each other, nor did sulphur differ from the check. At the same level of significance however, pollen germination in the dichlone, ferbam, and captan treatments was less than in the check and captan almost entirely prevented the germination of pollen. Figure 2 illustrates the effect of these materials on pollen germination.

Pollen Germination with Several Concentrations of Captan

In this experiment, replicated five times, captan was applied at 2 pounds/100 gallons, 0.2 pound/100 gallons, 0.02 pound/100 gallons, and 0.002 pound/100 gallons. The checks were sprayed with tap-water. The results are presented in Table 2. They clearly indicate a great reduction in the germination of pollen in the treatments at 2 pounds/100 gallons. In the other treatments and the checks, however, there were no significant differences between the average percentages of germination. The relatively low percentage of germination in the check in replicate 5 is likely due to imperfect cleaning of the equipment following the spraying of captan at 2 pounds/100 gallons in replicate 4.

Effect of Captan on Pollen-tube Elongation

In the experiments described above, the fungicides were sprayed immediately following the application of pollen to the slide. This experiment, on the other hand, was designed to study possible effects of captan on pollen that had already begun to germinate. It was repeated three times. Pollen was allowed to germinate for 45 minutes before being sprayed with tap-water as a check. Pollen was also allowed to germinate for periods of 45, 60, and 75 minutes before being sprayed with captan at 2 pounds/100 gallons. The check was photographed after 45 minutes' incubation and at 15-minute intervals thereafter until the end of the experiment. Captan treatments were photographed 2 minutes before being sprayed and at 15-minute intervals after spraying until the end of the experiment.

Figure 3, illustrating one of three replicates, clearly shows that pollen-tube elongation was arrested by 2 pounds/100 gallons captan. This was true whether the pollen had been allowed to germinate 45, 60, or 75 minutes prior to treatment.

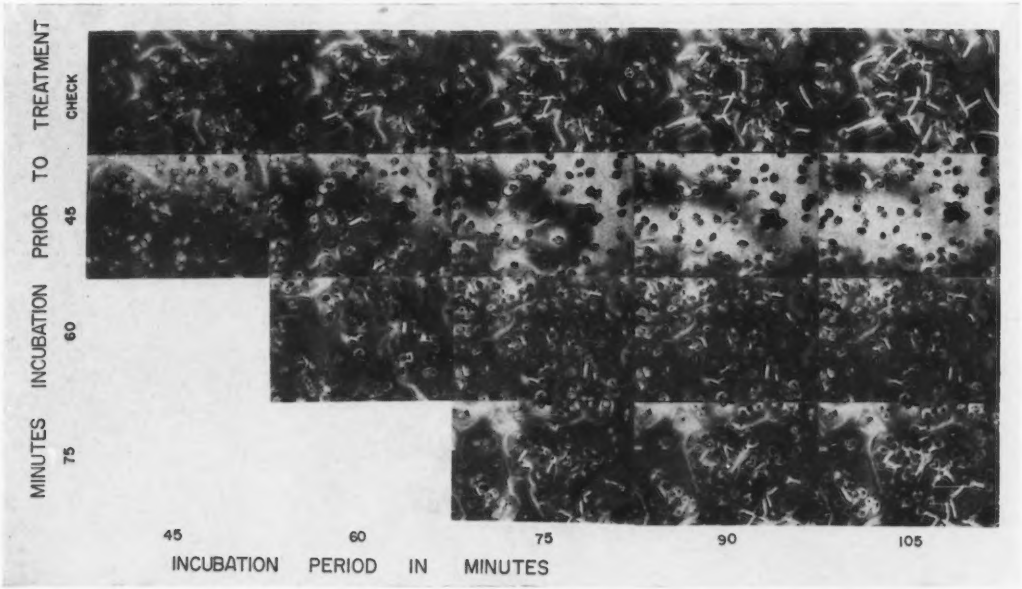

FIGURE 3. Germinating sweet cherry pollen (*top row*), and that allowed to germinate 45, 60, and 75 minutes before arresting with 2 pounds/100 gallons captan (*bottom three rows*). Check first photographed after 45 minutes' incubation, treatments when sprayed. Subsequent photographs at 15-minute intervals until, after 105 minutes' incubation, the end of the experiment. (32X.)

DISCUSSION

In the experiments reported here, sulphur had no significant effect on pollen germination. Dichlone and ferbam both significantly reduced pollen germination as compared with checks, but captan almost entirely prevented germination.

Differences between fungicides were not found by Rich (2) but were found in the experiments reported here. The reason is probably that different methods were used for testing the materials. Rich (2) attempted to germinate apple pollen in media containing the fungicides in the concentrations used in sprays. This author used the fungicides as sprays, rather than as constituents of the media, in an attempt to simulate natural conditions more closely. As a result, the concentrations of fungicides in the artificial media here were almost certainly somewhat less than in those of Rich (2).

In view of the proportion of pollen which germinated in dichlone and ferbam treatments, it seems likely that these materials and sulphur are not a hazard to fruit set*. The effects of captan on pollen germination merit further study under orchard conditions, particularly for the sweet cherry, and possibly for other fruits as well.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the technical assistance of M. G. Howard in much of this work. F. Edwards developed the photographic prints and R. G. Mercier performed the statistical analyses.

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*From advice given by G. C. Chamberlain, Canada Department of Agriculture, Research Laboratory, Vineland and St. Catharines, Ontario, and Extension personnel, the author concludes that captan is not necessary for control of blossom blight, since either dichlone or sulphur or a combination of these two gives better control. Because of the results herein reported, and the existence of superior materials, the use of captan during bloom would appear unwise until further information is available.

CHEMICAL CONTROL OF SNOW MOULD OF GRASSES AND ALFALFA IN ALBERTA[1]

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[Received for publication March 6, 1961]

ABSTRACT

Chemical tests for control of snow mould of turf grass and alfalfa were conducted at Edmonton from 1945 to 1957. Of a number of chemicals tested, inorganic mercury salts provided the best control. Mercuric chloride was more effective than mercurous chloride for treating alfalfa but no difference in control was observed from the two chemicals on turf grass. Sodium tetraborate sprays applied to the foliage of alfalfa in early fall gave remarkable control of the disease but are not recommended because of the low margin of safety from boron injury.

INTRODUCTION

Snow mould on forage crops, winter cereals, and turf grass has been under investigation for many years in North America, Scandinavia, and Japan. The most common of the low-temperature fungi causing the disease in these areas are *Sclerotinia borealis* Bub. and Vleug., *Fusarium nivale* (Fr.) Ces., and *Typhula* spp. (5, 7, 10, 11). *S. borealis* causes severe damage to forage crops in Alaska, Yukon, and northern British Columbia (7) and an unidentified low-temperature basidiomycete (2, 4), not reported yet in other countries, is widespread in Western Canada and causes most of the snow mould damage. *F. nivale* also occurs in Western Canada but is of minor or local importance.

Fungicides that prove satisfactory in controlling snow mould in one geographical region are often ineffective in others, presumably due to differences in climate and the pathogens involved. For example, Jamalainen (6) reported effective control of snow mould on winter wheat and winter rye in Finland with pentachloronitrobenzine, whereas Sprague (10) was unable to control the disease on winter wheat with the same chemical in Washington.

Chemical treatment for controlling snow mould in Canada is confined mainly to turf grass on golf- and bowling-greens. The high cost of effective chemicals makes their use uneconomical for forage crops or golf-course fairways, and the mammalian toxicity makes the treatment of private lawns hazardous. The construction of new golf-courses and the conversion of greens from sand to grass on established courses in Canada have greatly increased the culturing of grass species that are highly susceptible to snow mould. Consequently, there is a greater need for a more economical and effective method of controlling the disease.

This paper summarizes the results obtained in Edmonton, Alberta, from chemical treatments on alfalfa and grass for control of snow mould caused by the low-temperature basidiomycete.

MATERIALS AND METHODS

Chemical tests for snow mould control were conducted on experimental plots, 16 feet square or larger, previously infested with the low-temperature

[1]Joint contribution from the Canada Department of Agriculture Research Stations, Lethbridge, Alberta, and Saskatoon, Saskatchewan, and the Plant Pathology Laboratory, Edmonton, Alberta.

basidiomycete using the method described by Cormack (4). The chemicals were either mixed with fine sand as a carrier and applied at the rate of 10 quarts per 1000 square feet or as a spray with water as a diluent and applied at the rate of 6 gallons per 1000 square feet. Each treatment was replicated at least three times. The chemicals were applied in the fall and percentage survival in the plots was estimated and recorded the following spring.

The ingredients of the compounds used in these trials were: Acti-dione (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide; borax (sodium tetraborate); Cadminate (60 per cent cadmium succinate); Calo-Clor (60 per cent mercurous chloride and 30 per cent mercuric chloride); Calo-Cure (30 per cent mercurous chloride and 15 per cent mercuric chloride); Ceresan (2-methoxyethyl mercuric chloride); Dyrene (2,4-dichloro-6-(O-chloroaniline)-S-triazine); Panogen (6.3 per cent methylmercuric cyanoguanidine); P.M.A.S. (10 per cent phenyl mercuric acetate); Puraturf powder and Puraturf liquid No. 10 (6 per cent phenylmercury-triethanol ammonium); Phygon (2,3-dichloro-1:4-naphthoquinone), and special Semesan (24 per cent hydroxymercuric-chlorophenol and 2 per cent hydroxymercuric cresol).

RESULTS

Fungicidal Treatments of Alfalfa

The chemicals were applied in the fall to alfalfa plots infested with the low-temperature basidiomycete. In preliminary experiments the chemicals were placed near the crowns of inoculated plants in rows; subsequently, they were applied to plots of alfalfa replicated four times. The plots measured 16 feet square or larger. A count or estimate of survival in the alfalfa was made the following spring. As shown in Table 1, survival was higher in plots treated with the inorganic mercury compounds than with Ceresan and special Semesan. Mercuric chloride (corrosive sublimate) was

TABLE 1. — SURVIVAL OF ALFALFA IN PLOTS INFESTED WITH THE LOW-TEMPERATURE BASIDIOMYCETE AND TREATED WITH VARIOUS CHEMICALS IN THE LATE FALL, EDMONTON, 1945-48

Chemical	Treatment	Survival, per cent			
		1945-46	1946-47	1947-48	Av.
Untreated	—	0	0	0	0
Mercuric chloride	Soil[1]	90	90	95	92
Mercurous chloride	Soil[1]	55	40	80	58
Ceresan	Soil[1]	30	45	55	43
Special Semesan	Soil[1]	35	25	55	35
Borax	Soil[2]	30	50	20	33
Borax	Foliage spray[3]	70	90	65	75

[1]4 oz. per 1000 per sq. ft.
[2]50 oz. per 1000 per sq. ft.
[3]One application of 1.5% solution

consistently more effective than mercurous chloride (calomel) in controlling snow mould in alfalfa. In experiments with other materials survival of alfalfa was low (10-25 per cent) in plants treated with P.M.A.S., Phygon, and zinc chloride (all at 4 ounces per 1000 square feet). There was little or no survival in plots treated with lime (1000 pounds per acre), sulphur (1000 pounds per acre), urea (500 pounds per acre), copper sulphate, copper acetate, and cuprous oxide (all at 4 ounces per 1000 square feet).

Boron Treatments of Alfalfa

Boron treatments reduced snow mould damage in alfalfa in preliminary tests and a study was made on the effect of variation of methods and time of application. Commercial borax was applied to the soil as a powder or drench at rates ranging from 10 to 50 ounces per 1000 square feet at seeding time and at various times in the fall. In spray treatments boron solutions were uniformly applied with a knapsack sprayer to the tops of the plants at various times in the fall.

Foliage sprays with boron were much more effective than soil treatment in controlling snow mould of alfalfa (Table 1). This was also demonstrated with plots of alfalfa inoculated and assessed by the method described by Cormack (4). The average spread of killing in three experiments was 1.1 inches in plots sprayed with boron, 2.5 inches after soil treatments with boron, and 2.8 inches in the untreated controls.

In an experiment on time and rate of spraying (1947-48) the average survival of alfalfa in inoculated blocks (16 square feet, four replicates) was as follows:

Concentration of borax	Date of application	Average survival
%		%
1.5	Sept. 11 and 22	90
1.5	Sept. 22	65
1.5	Sept. 11	65
0.4	Sept. 11	40
Control	—	5

The degree of control with two successive sprayings in this experiment is illustrated in Figure 1 and was nearly equal to the average of that achieved by soil treatment with corrosive sublimate in the experiments summarized in Table 1. Survival in the boron-sprayed plots varied from year to year. In 1948-49 it was highest (40 per cent) in plots that received three successive sprays (September 10, 20, and 30) and lowest (25 per cent) in plots sprayed on September 1. Early fall treatments generally had less effect than those applied later; however, with late spraying the tops were sometimes frozen shortly afterward and the treatment had little or no effect. Commercial borax gave as good results as boric acid and several other formulations of boron. Various spreaders, stickers, or other additives, including detergents, had no effect on the treatments. Most spray solutions were prepared with a 0.02 per cent aerosol solution.

Boron injury (chlorosis of leaf margin) was evident in the early spring growth in all plots that received sufficient spray to control snow mould.

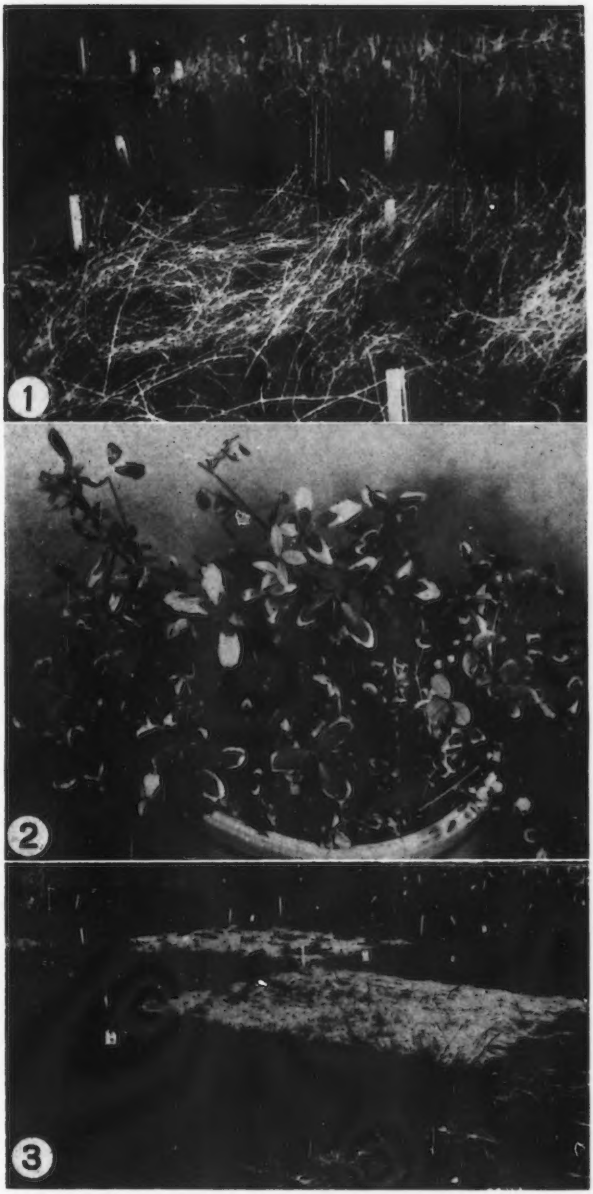

FIGURE 1. Snow mould of alfalfa controlled by two sprayings of the foliage with 1.5 per cent borax in September. All plants have been killed in the untreated plot in the foreground.

FIGURE 2. Symptoms of boron injury on foliage of alfalfa plants sprayed with a 1.5 per cent solution of borax.

FIGURE 3. Control of snow mould on turf grass infested with the low-temperature basidiomycete. Plot in foreground treated with Calo-Clor (3 oz. per 1000 sq. ft.) is contrasted with the untreated adjoining plot.

Later growth was not obviously affected. Injury was also observed shortly after spraying in the fall, especially in plots that received more than one application. Similar symptoms of boron injury developed on alfalfa plants sprayed with borax solutions in the greenhouse (Figure 2).

Fungicidal Treatments of Turf Grass

Inorganic mercury compounds are generally used and prove to be effective fungicides against snow mould organisms prevalent in Canada (Figure 3). A mixture of corrosive sublimate and calomel is generally recommended (1) and is sold under several trade names. Commercial companies merchandise these two chemicals in mixtures on the assumption that the more soluble corrosive sublimate is readily available to control snow mould in the fall, whereas calomel, being sparingly soluble, persists longer and is more effective in the spring.

To test the validity of this hypothesis the following experiment was conducted. Plots (8 feet square) of *Agrostis tenuis* L. infested with snow mould were treated in the fall. Calomel and corrosive sublimate were applied at the same rate (mercury equivalent) and at half the rate of the mixture (Calo-Clor). The treatments were replicated three times and the plots were rated for survival the following spring. The results (Table 2) provide no evidence to support the view that a combination of the two forms of mercury is more effective than either one used alone. This confirms an earlier report by Broadfoot (1).

Other experiments were conducted to find fungicides just as effective but more economical than inorganic mercury salts. The comparative control obtained from these chemical treatments is shown in Table 3. Only Calo-Clor gave effective control. Cadminate and phenol mercury preparations have given good results in the Pacific Northwest (9) but were ineffective in these trials. Panogen gave better control of the disease at 6 ounces per 1000 square feet than at 3 ounces. This suggested that equally good control might be obtained with an organic compound such as Panogen, if the dosage were increased to a level approaching the mercury equivalent of that used in the inorganic compound, Calo-Clor. Subsequent tests, however, showed that survival in the plots declined when rates higher than

TABLE 2. — SURVIVAL OF *AGROSTIS TENUIS* TREATED WITH MERCURY COMPOUNDS FOLLOWING INFESTATION WITH THE LOW-TEMPERATURE BASIDIOMYCETE, EDMONTON, 1957

Chemical	Dosage per 1000 sq. ft.	Survival
	ounces	per cent
Untreated	—	50
Calo-Clor (mercuric chloride + mercurous chloride)	3.00	86
Mercuric chloride	1.40	86
Mercuric chloride	2.81	90
Mercurous chloride	1.32	90
Mercurous chloride	2.64	96

TABLE 3. — SURVIVAL OF *AGROSTIS TENUIS* TREATED WITH VARIOUS FUNGICIDES FOLLOWING INFESTATION WITH THE LOW-TEMPERATURE BASIDIOMYCETE, EDMONTON, 1955-57

Fungicide	Dosage per 1000 sq. ft.	Survival
		per cent
Untreated	—	0
Cadminate	3 ounces	0
Borax	20 "	6
Panogen	3 "	8
P.M.A.S. 10%	2 "	12
Puraturf liquid No. 10	2 "	13
Acti-dione	1 gram	20
Calo-Cure	3 ounces	25
Dyrene	11 "	30
Puraturf powder	3 "	38
Panogen	6 "	55
Calo-Clor	3 "	77

6 ounces of Panogen per 1000 square feet were used. The same effect was observed when the dosage of P.M.A.S. was increased appreciably beyond the rates reported in Table 3. The decline of survival in the plots when treated with these higher rates was presumably due to the phytotoxicity of these chemicals. No investigation was made on the phytotoxic thresholds of any of these fungicides. Dosages were derived from those generally recommended by the manufacturer. Acti-dione had little effect on the development of snow mould under field conditions even though it shows a distinct antibiotic effect on the organism in the laboratory (12).

Boron was included in the test because of the effective control it gave on snow mould of alfalfa when used as a foliage spray but it gave practically no control of the disease on turf grass.

DISCUSSION

Under conditions in Western Canada the inorganic mercury salts were superior to all other chemicals tested for control of snow mould. These compounds appear to be just as effective in either soluble or sparingly soluble forms on turf grass; however, on alfalfa the more soluble form gave the best control.

The ineffectiveness of phenol mercury and Cadminate preparations in controlling snow mould in Alberta as compared with the Pacific Northwest may be due to the differences in climatic conditions and causal organisms.

Foliage spray treatments of boron are not recommended for the control of snow mould in alfalfa, mainly because of the low margin of safety from boron injury. This injury is most evident in the early spring growth and it might be accentuated by repeated treatments or by the use of excessive amounts of boron. Also, the effectiveness of boron sprays in controlling snow mould appears to be greatly influenced by seasonal conditions and other factors. Sprays should be applied to the green foliage as late as possible in the fall. However, an early frost may destroy the tops before

the spraying date or nullify the results if freezing occurs shortly afterwards. If these difficulties could be overcome, foliage sprays with commercial borax should be an economical method of controlling snow mould in alfalfa.

No definite information has yet been obtained on the nature of the protective action against snow mould provided by boron foliage sprays. The results suggest that boron solutions absorbed by the foliage may act indirectly by altering the physiology of the plant so that it can withstand the action of the pathogen. It has been shown that disease development is correlated with the accumulation of hydrogen cyanide in the affected tissues (8). Recent work (3) indicates that this toxic substance is produced by the interaction of an enzyme secreted by the fungus and a cyanogenetic substrate present in the host. Necrosis presumably occurs after inhibition of the respiratory enzymes of the host by free hydrogen cyanide. It is possible that boron absorbed by the foliage either interferes with this enzymic release of hydrogen cyanide or prevents the blockage of the respiratory enzyme system. Further investigation of these processes may provide information that will eventually lead to the control of this disease.

Continued search should be made for chemicals that will give equally good or better control of snow mould on turf grass than the expensive inorganic mercury compounds. More information is also needed to determine the most efficient methods and times for applications of fungicides. Based on present knowledge the best control in Western Canada is obtained by growing the most resistant grass available and applying mercuric or mercurous chloride at 3-4 ounces per 1000 square feet late in October, preferably before the first heavy snowfall.

Crop rotation and culturing resistant varieties are the two important control measures for snow mould on forage crops. Chemical treatments are still too costly to be practical in controlling low-temperature pathogens on these crops.

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THE INFLUENCE OF SEEDING RATE ON THE YIELD AND THE YIELD COMPONENTS OF WHEAT, OATS AND BARLEY[1]

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[Received for publication February 8, 1961]

ABSTRACT

The response of two wheat, three oat and three barley varieties to six seeding rates was measured at Beaverlodge, Fort Vermilion and McLennan for 3 years. Seeding rates of 1.5 bushels per acre for wheat and 2.5 bushels for oats give optimum yields at all locations. For barley, a rate of 2.0 bushels per acre is optimum at Beaverlodge and Fort Vermilion, but at McLennan, because of reduced emergence, a rate of 2.5 bushels is required. Variety and season do not alter appreciably the influence of seeding rate on yield.

There are well defined locational and varietal differences in yield and in the four components of yield, namely (i) number of plants per acre, (ii) number of fertile heads per plant, (iii) number of kernels per head, and (iv) weight per 1000 kernels. These components explain certain locational and varietal yield responses but do not provide an absolute index of yielding ability.

For all crops, the increase in seeding rate causes a linear increase in the number of plants per acre and a curvilinear decrease in the number of fertile heads per plant. There are also associated reductions in the number of kernels per head and the 1000-kernel weight, but the type of response varies with crop.

Certain inadequacies are suggested in the present methods of selecting and testing varieties and hybrids of these crops for yield.

INTRODUCTION

The yield of an individual plant of wheat, oats or barley is the product of (i) the number of heads, (ii) the number of kernels per head, and (iii) the average weight of the kernels. These are reported to be influenced by variety (2, 3, 4), location (4), date of seeding (2), and level of soil nitrogen (3). However, when grown in the field, the individual is obscured by the population and yield is based on the weight of seed from a given area. This introduces an additional component (iv), the number of plants per unit area which, unless altered by reduced viability or restricted emergence, is a direct function of seeding rate. As such, it may condition the response of the other three components without necessarily influencing yield (1).

Studies have determined the influence of seeding rate on yield but have ignored essentially the influence of rate on the yield components of the individual plants. Conversely, determinations of the yield components have not been based on planned variations in plant density. The present study was designed to determine the influence of seeding rate on population yields and on the yield components of the individual plants.

MATERIALS AND METHODS

The response of Saunders and Thatcher wheat, Ajax, Beaver and Victory oats, and Newal, Olli and Vantage barley to six seeding rates was measured at three locations for 3 years. When the study was initiated these varieties were considered to be the most suitable for the region. With

[1]Joint publication from the Experimental Farms at Beaverlodge and Fort Vermilion, Alberta.
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[4]Agronomist (Grey Wooded Soils), Experimental Farm, Beaverlodge, Alta.

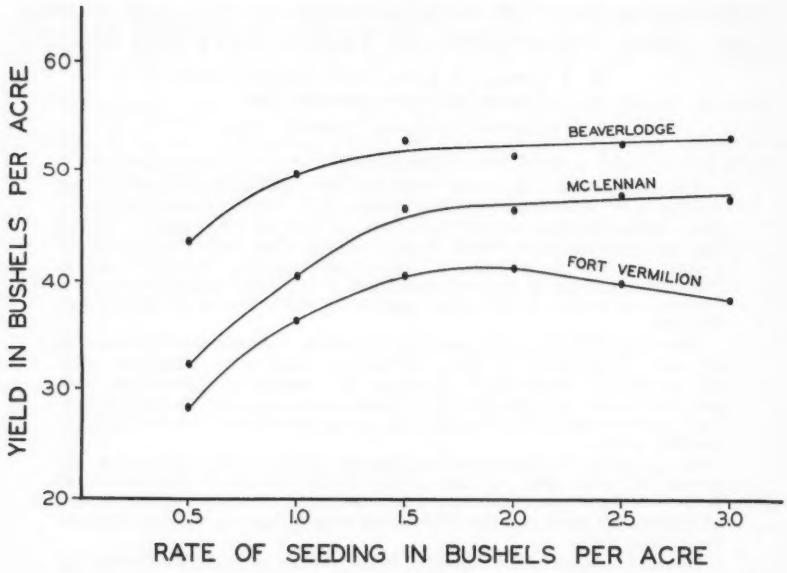

FIGURE 1. The influence of seeding rate on the yield of wheat at three locations.

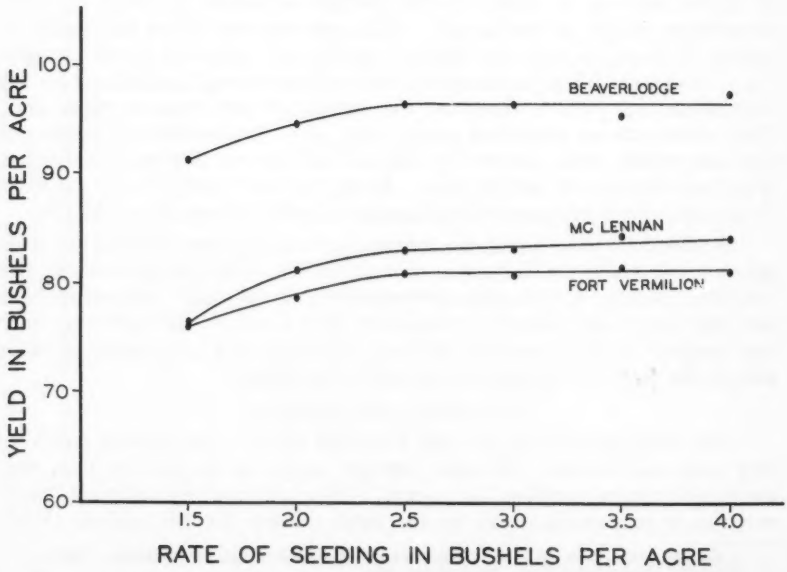

FIGURE 2. The influence of seeding rate on the yield of oats at three locations.

the exception of the wheat varieties, they had been found to differ in yield and in at least one of the yield components.

Tests were conducted on Degraded Black soils at the Experimental Farms at Beaverlodge and Fort Vermilion and on Grey Wooded soil at the Experimental Project Farm at McLennan. All crops were compared at Beaverlodge and McLennan during 1954 to 1956, inclusive. At Fort Vermilion wheat and barley were compared during 1956 to 1958, inclusive, and oats in 1956, 1957 and 1959. All tests were seeded on summerfallow using a 6-inch spacing between rows. No fertilizer was used at Fort Vermilion but ammonium phosphate (11-48-0) at 25 pounds per acre was applied with the seed at Beaverlodge and McLennan. This conformed with the cultural recommendations for the areas at the time the study was initiated.

Seeding rates were based on the statutory weight per bushel for each crop. They ranged in 0.5 bushel per acre increments from 0.5 to 3.0 bushels for wheat, from 1.5 to 4.0 bushels for oats, and from 1.0 to 3.5 bushels for barley. A split-plot design was used for each crop with varieties as main plots and rates as subplots. Each subplot consisted of four rod-rows and was replicated four times.

The yield in bushels per acre, number of plants per acre, number of fertile heads per plant, number of kernels per head, and 1000-kernel weight were determined for the two centre rows of each subplot. The yield in bushels per acre and 1000-kernel weight were calculated from the threshed sample. Number of plants per acre was derived from a plant count taken immediately after complete emergence. Number of fertile heads per plant was obtained by dividing the total number of fertile heads, just prior to harvest, by the number of plants that emerged. Number of kernels per head was calculated from the plot yield in grams, the 1000-kernel weight and the total number of fertile heads per plot.

RESULTS AND DISCUSSION

Yield

At the lower seeding rates there were usually significant increases in the yield of wheat with increases in seeding rate and consistent but generally non-significant increases in the yield of oats and barley. The influence of seeding rate on yield was not altered appreciably by variety or season. Because of this, the data were averaged to determine the influence of seeding rate on the yield of each crop at each location. Accordingly, data from the two varieties and 3 years were averaged to obtain the wheat yields in Figure 1 and data from three varieties and 3 years averaged for the oat and barley yields presented in Figures 2 and 3, respectively.

Seeding rates of 1.5 bushels per acre for wheat and 2.5 bushels per acre for oats are optimum at all locations despite locational variations in the level of yield. As shown in Figure 3, the relationships are not as precise for barley. At Beaverlodge and Fort Vermilion the seeding rate of 2.0 bushels per acre is optimum while at McLennan the optimum rate approaches 2.5 bushels.

TABLE 1. — AVERAGE LOCATIONAL YIELDS AND YIELD COMPONENTS FOR WHEAT, OATS AND BARLEY

Yield and yield components	Wheat			Oats			Barley		
	Beaverlodge	McLennan	Fort Vermilion	Beaverlodge	McLennan	Fort Vermilion	Beaverlodge	McLennan	Fort Vermilion
Yield in bushels per acre	50.6	43.6	37.5	95.2	82.1	79.8	57.7	61.5	58.7
No. of plants per acre[1]	1.2	0.9	1.0	1.2	1.0	1.1	1.1	0.9	1.2
No. of fertile heads per plant	1.9	2.7	1.9	1.2	1.9	1.6	1.2	1.8	1.3
No. of kernels per head	23.4	19.9	18.2	34.4	25.4	26.2	29.1	28.4	23.2
Weight per 1000 kernels in grams	35.3	31.5	35.3	33.2	31.9	30.6	37.3	36.2	39.4

[1]Recorded as millions of plants per acre

TABLE 2. — AVERAGE VARIETAL YIELDS AND YIELD COMPONENTS FOR WHEAT, OATS AND BARLEY

Yield and yield components	Wheat		Oats			Barley		
	Saunders	Thatcher	Ajax	Beaver	Victory	Newal	Olli	Vantage
Yield in bushels per acre	44.3	43.6	83.0	81.7	92.4	56.9	57.9	63.0
No. of plants per acre[1]	1.0	1.1	1.2	1.0	1.1	1.0	1.2	1.0
No. of fertile heads per plant	2.1	2.2	1.7	1.7	1.4	1.4	1.4	1.5
No. of kernels per head	20.7	20.4	26.9	26.2	33.1	25.7	28.0	27.0
Weight per 1000 kernels in grams	35.8	32.2	29.0	33.8	33.0	39.7	32.4	40.8
Average weight per 1000 kernels in grams of seed sown	37.3	33.5	30.9	34.9	33.6	39.3	33.8	40.9

[1]Recorded as millions of plants per acre

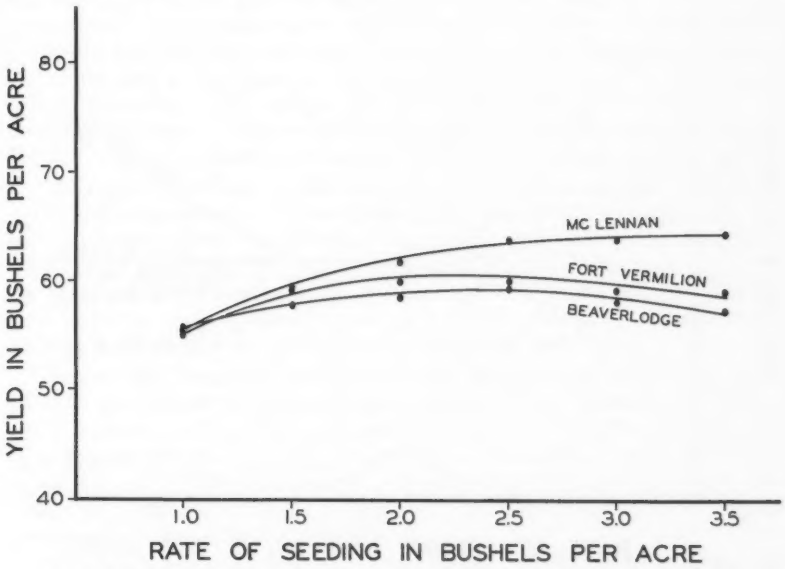

FIGURE 3. The influence of seeding rate on the yield of barley at three locations.

With increases in seeding rate the yield of oats increases up to the optimum rate and is maintained with further increases at all locations. Wheat is somewhat less stable in that there are small further increases in yield at Beaverlodge and McLennan and sharp reductions in yield at Fort Vermilion when the seeding rate surpasses the optimum. Barley has the least stable yield. There are yield increases associated with seeding rates of up to 3.5 bushels per acre at McLennan, resulting from the reduced emergence caused by crusting of the soil surface. However, at Beaverlodge and Fort Vermilion yield depressions are caused by above optimum seeding rates for barley.

Yield Components

The average yields for locations and for varieties, and the corresponding averages for number of plants per acre, number of fertile heads per plant, number of kernels per head, and weight per 1000 kernels in grams are presented in Tables 1 and 2, respectively. Location averages for wheat are the mean of six seeding rates for two varieties during 3 years and for oats and barley are the mean of six seeding rates for three varieties during 3 years. Variety averages are based on six seeding rates at three locations during 3 years.

As shown in Table 1, there are well defined locational variations in the yield components. For all crops, the number of plants per acre is lower at McLennan than at Beaverlodge and Fort Vermilion because of reduced emergence resulting from surface soil crusting prior to emergence. How-

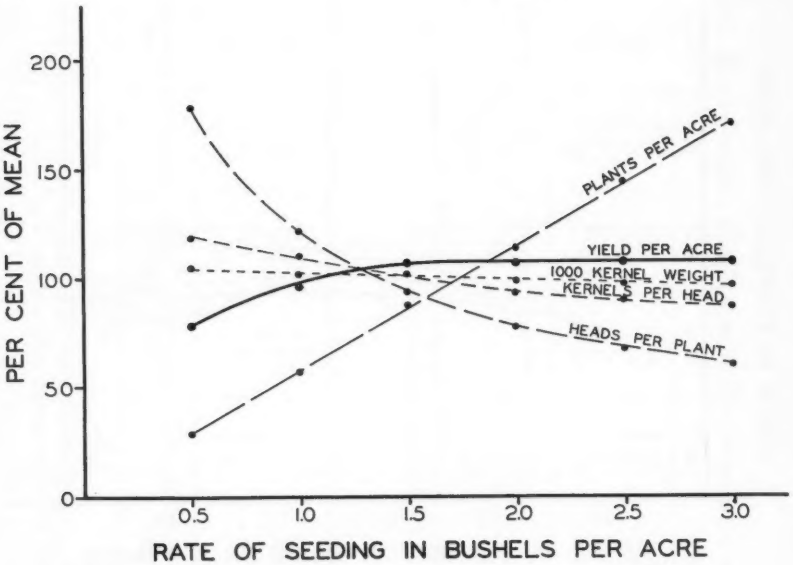

FIGURE 4. The influence of seeding rate on the yield and the yield components of wheat.

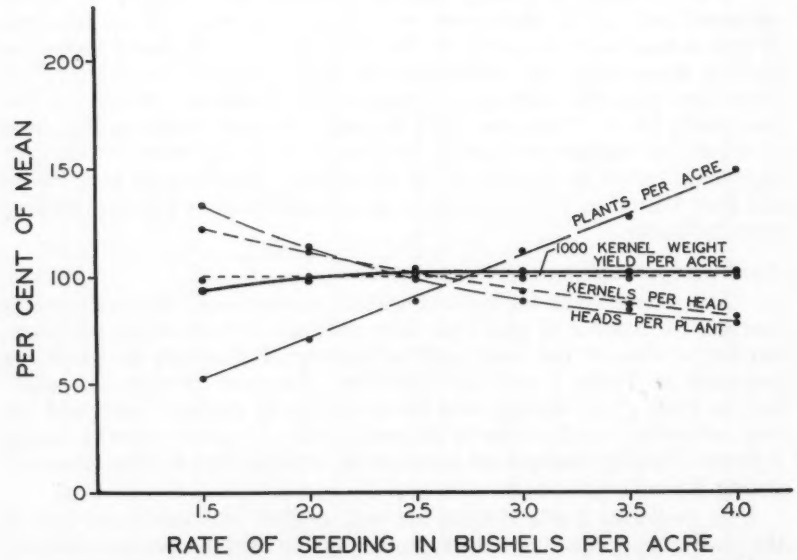

FIGURE 5. The influence of seeding rate on the yield and the yield components of oats.

ever, the influence on yield of this reduction in plant number is more than compensated for by an increase in the number of fertile heads per plant. For wheat and oats there are fewer kernels per head at McLennan and Fort Vermilion than at Beaverlodge, but for barley, kernel number is lower only at Fort Vermilion. The weight per 1000 kernels tends to be lower for all crops at McLennan. The components explain certain locational differences in yield but do not provide an absolute index of locational yielding ability.

As shown in Table 2, there are also well defined varietal differences in the yield components. Since seeding rate was based on weight, the inherently larger seeded varieties had fewer plants per acre. However, this reduction in plant number does not appear to directly influence tillering or any of the other yield components. This suggests that in yield tests seeding rates based on a constant weight of seed should provide as suitable comparisons as rate based on a given number of kernels per row.

Despite the locational and varietal differences in the four yield components, the relative response of each component to variations in seeding rate is similar at all locations and for all varieties. Therefore, all data for each crop are averaged to assess the general response of the yield components to seeding rate. The relationships are plotted in Figures 4, 5 and 6 for wheat, oats and barley, respectively, with each rate presented as a percentage of the mean for the component.

The response to seeding rate of the number of plants per acre and of the number of fertile heads per plant are similar for wheat, oats and barley.

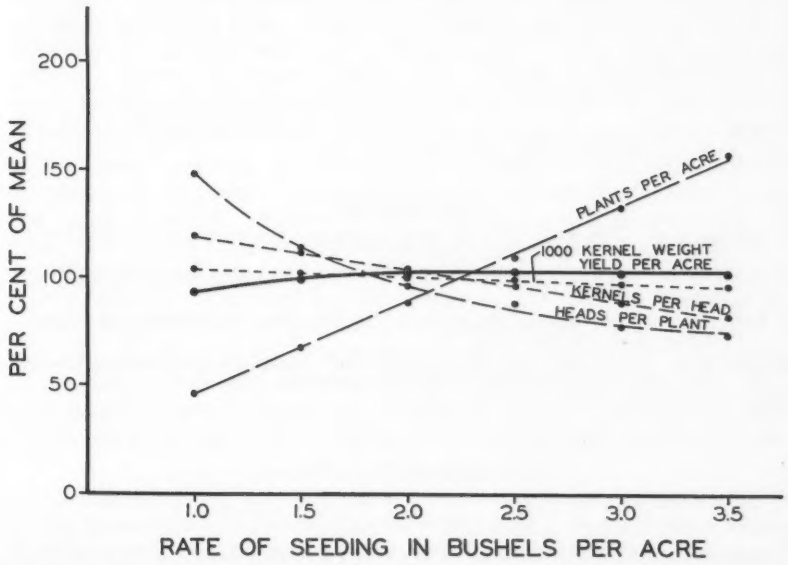

FIGURE 6. The influence of seeding rate on the yield and the yield components of barley.

The number of plants per acre is a direct, positive, linear function of seeding rate. This increase in plant number is accompanied by a strong, curvilinear decrease in the number of fertile heads per plant. These two components have the greatest influence on yield.

Number of kernels per head and 1000-kernel weight are not as greatly influenced by seeding rate and the response is conditioned by crop. For wheat, increase in seeding rate causes a moderate, curvilinear decrease in the number of kernels per head and a smaller, linear decrease in the 1000-kernel weight. For oats, there is only a strong, curvilinear decrease in the number of kernels per head, seeding rate having no influence on the 1000-kernel weight. This reduction in the number of kernels per head could be associated with the blasting usually evident in heavy stands of oats. Increase in seeding rate for barley is accompanied by moderate, linear decreases in both the number of kernels per head and in the 1000-kernel weight.

As well as explaining the influence of seeding rate on the yield of wheat, oats and barley, the interactions of these components suggest the inadequacy of the present method of individual plant selection from space planted, early generation hybrid material. Because of the strong interaction between number of plants per acre and number of heads per plant, there is little value in using tillering as a selection index unless spacing and fertility are uniform or the spacing is sufficiently wide to allow all plants to express their maximum tillering potential. However, it does not seem practical to allow for maximum tillering when at optimum seeding rates wheat, oats and barley do not normally produce more than an average of one tiller. It appears, then, that selection efficiency for yield might be increased by seeding hybrid material sufficiently heavy to eliminate tillering and by selecting individual heads on the basis of the number of kernels per head and 1000-kernel weight. This must presume, of course, that the parents used will transmit to the hybrids sufficient tillering potential for normal adjustment to irregular seeding and slightly sub-optimum seeding rates that may be encountered in the field.

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AWN BRITTLENESS IN BARLEY[1]

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[Received for publication April 21, 1961]

ABSTRACT

Awn brittleness measurements were made on barley varieties grown at three locations in the Atlantic Provinces and Brandon, Manitoba, by a method previously described by the author. Statistically significant differences in brittleness between varieties were obtained on samples from each location. The agreement in ranking of varieties at the three Atlantic locations was highly significant. Varieties grown at Brandon, Manitoba, and Fredericton, New Brunswick, showed only small differences in brittleness due to location. Tests conducted at Brandon gave considerably higher brittleness percentages than tests at Fredericton, irrespective of where the crop was grown. It is concluded that the method gives a reliable measure of awn brittleness, but environmental conditions during storage of samples and testing would have to be controlled to permit similar results to be obtained under varying conditions.

INTRODUCTION

The difficulty of removing awns from some barley varieties during threshing is a well-known problem in the Atlantic Provinces. Varieties, otherwise well adapted, have been rejected because of this difficulty. The most extreme example is probably the attempts to grow winter barley in the Annapolis Valley of Nova Scotia. Several years of testing winter barley in that area showed that some varieties were well adapted. When field trials were attempted, it was found impossible to thresh this barley satisfactorily.

While this has been regarded as a problem peculiar to the Atlantic Provinces there are reports that some varieties are troublesome in other areas. Platt and Wells (4) refer to Titan barley as being very difficult to thresh in Alberta. Pope (5) describes barley varieties grown in Virginia as having exceedingly tough adherent awns.

Few workers have attempted to measure awn brittleness in barley. While Harlan and Pope (3), Pope (5), and Aberg (1) have dealt with the association of ash content in awns with brittleness, they do not refer to any method of measuring. Apparently their conclusions were based on observations of threshed samples. Platt and Wells (4) counted the number of kernels with awns attached in a 100-kernel sample of barley threshed in a Kemp thresher. A method has been suggested by Chiasson (2) whereby the brittleness of awns on individual heads is measured.

The objectives of the present study were: (a) to classify varieties and strains according to their awn brittleness; (b) to determine the awn brittleness of varieties and strains grown at different locations; (c) to obtain additional data pertaining to the reliability of the method described by Chiasson.

MATERIALS AND METHODS

Measurements of awn brittleness were made on varieties and strains of spring barley grown at Fredericton, New Brunswick; Nappan, Nova Scotia; Charlottetown, Prince Edward Island; and Brandon, Manitoba. Samples of

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winter barley grown at Kentville, Nova Scotia, were also tested. The samples were collected from the border rows of regular yield tests and stored in a heated room until tested.

The percentage of brittle awns was obtained from eight spikes, picked at random from each replication. There were six replications in each test, except in the winter barley test where only four replications were used. The tests were all conducted at Fredericton, except for one test on four varieties which was conducted at Brandon, Manitoba.

The data were transformed to arcsin $\sqrt{\text{percentage}}$ values. Duncan's Multiple Range Test ($P = .05$) was applied to the transformed data. The mean of each variety was then re-transformed to a percentage value. Kendall's coefficient of concordance (6) was calculated from the ranking of the varieties in the three tests of spring barley conducted in the Atlantic Provinces.

RESULTS AND DISCUSSION

It is evident from the data presented in Table 1 that there are significant differences in awn brittleness between varieties and strains. There was considerable similarity in the performance of varieties at the three locations as the coefficient of concordance was significant at the 1 per cent level of probability. It may be concluded that a satisfactory separation of the varieties on the basis of awn brittleness has been effected by this method.

No comparison between the awn brittleness of varieties as measured by this method and their performance under actual threshing conditions has been attempted. However, previous experience with some of the varieties

TABLE 1. — PERCENTAGE BRITTLE AWNS IN SPRING BARLEY VARIETIES AND STRAINS

Fredericton, N.B.		Nappan, N.S.		Charlottetown, P.E.I.	
Variety	per cent	Variety	per cent	Variety	per cent
M.C. 247	95.5[1]	M.C. 247	96.5	M.C. 247	96.9
York	94.7	5088-8-41	92.8	Len 30	90.6
5088-8-4-1	91.0	York	91.9	M.C. 2148	86.9
M.C. 2148	86.4	M.C. 2148	90.9	York	82.4
O.B. 4-13	81.7	Len 30	83.6	M.C. 2850	78.3
Len 30	79.9	O.B. 13-10	80.6	5088-8-4-1	75.3
M.C. 2850	76.6	O.A.C. #21	75.5	O.B. 13-10	62.0
U.M. 1316	76.0	O.B. 4-13	70.7	5069-40	61.6
5069-40	75.2	O.B. 22-1	67.3	U.M. 1316	55.5
O.B. 13-10	71.2	M.C. 2850	67.2	O.A.C. #21	53.7
O.A.C. #21	71.0	M.C. 2550	67.0	O.B. 22-1	48.9
5069-142	68.4	5069-40	64.2	O.B. 4-13	48.8
M.C. 2550	64.7	U.M. 1316	64.0	Montcalm	46.8
O.B. 22-1	59.2	5069-142	58.3	O.B. 23-3	46.2
O.B. 23-2	49.0	O.B. 23-2	48.2	M.C. 2550	39.3
O.B. 23-3	30.1	Montcalm	44.4	5069-142	39.0
Montcalm	27.6	O.B. 23-3	38.3	O.B. 22-3	31.1
Fort	24.3	O.B. 22-3	22.3	O.B. 23-2	24.6
O.B. 22-3	13.5	Fort	8.8	Fort	13.4
Mean	66.7		66.5		58.3

[1]There are no significant differences between varieties within the groups indicated by vertical lines.

TABLE 2. — PERCENTAGE BRITTLE AWNS IN WINTER BARLEY GROWN AT KENTVILLE, N.S.

Variety	Per cent
Kennate	31.4
G.H. 8	25.2
G.H. 12	20.7
G.H. 5	15.0
G.H. 14A	11.4
Hudson	4.8
G.H. 10	4.1
G.H. 14B	3.2
G.H. 7	2.9

indicates a satisfactory agreement. The varieties Fort and Montcalm have been recognized in the Atlantic area as having tough awns while York has been considered to have brittle awns. Similar agreement between brittle awn measurements and threshability has previously been presented by Chiasson (2).

Table 2 shows the percentage of brittle awns in nine varieties of winter barley grown at Kentville, Nova Scotia. The percentage of brittle awns of the varieties indicates they can all be classed as tough. This is entirely in agreement with observations on the same varieties at Kentville, Nova Scotia, where they were found to have such tough awns that they could not be threshed satisfactorily. Nevertheless, there are significant differences in brittleness shown by Duncan's Multiple Range Test. This shows the method can measure differences between strains which are too small to have any practical significance and can therefore adequately pick out differences of practical significance. The results indicate also that this method would be of considerable value in foretelling the threshability of varieties. The varieties tested at Kentville would have been shown to be too tough-awned to be suitable for the area and could have been replaced by more suitable varieties.

A general impression among cerealists is that awn toughness is a more serious problem in the Atlantic Provinces than in most other areas of Canada. An attempt was made to determine if this was actually true and, if so, whether it was due to different growing conditions or otherwise. Table 3 shows the results obtained in awn brittleness tests conducted at Brandon and Fredericton on barley varieties grown at both locations. The data indicate that awn brittleness is only slightly influenced by the location at which the crop is grown. Differences in percentages of brittle awns were 3.0 and 3.3 in favour of the Fredericton-grown crop when tested at Fredericton and Brandon, respectively. The tests at Brandon, however, averaged 63.2 and 66.5 per cent brittle awns compared to 39.6 and 42.6 per cent obtained at Fredericton.

No attempts were made to determine the cause of these differences. It seems very likely, however, that differences in atmospheric conditions prior to or during the tests must be responsible. If this is so it may well

TABLE 3. — PERCENTAGE BRITTLE AWNS IN BARLEY VARIETIES GROWN AND TESTED AT BRANDON, MANITOBA, AND FREDERICTON, NEW BRUNSWICK

Variety	Brandon readings grown at		Fredericton readings grown at	
	Brandon	Fredericton	Brandon	Fredericton
Montcalm	60.9	56.3	21.0	28.3
Lennoxville 30	47.4	61.1	32.2	41.3
Fort	50.0	59.7	25.5	26.0
U.M. 1316	94.7	88.9	79.7	74.8
Average	63.2	66.5	39.6	42.6

explain why more difficulties are encountered in removing awns in the Atlantic area than in other areas. It would also indicate that some standardization of environmental conditions will be required to obtain reasonably similar readings at different locations.

ACKNOWLEDGEMENTS

The author wishes to thank the following for their collaboration in collecting and forwarding samples used in these tests: D. R. Metcalfe, Experimental Farm, Brandon, Man.; H. H. Riordon, Nappan, N.S.; G. Smeltzer, Kentville, N.S., and R. B. MacLaren, Charlottetown, P.E.I.

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YIELD-MATURITY RELATIONSHIPS IN OATS[1]

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[Received for publication April 17, 1961]

ABSTRACT

Based on data collected over a period of 14 years from an average of 16 stations represented each year, and a total of 143 varieties tested, a positive correlation between maturity and yield is reported when conditions are favourable for all maturity classes. In the presence of stem and leaf rust, and under certain climatic conditions, late varieties were less productive.

The manner in which these results affect production and breeding is discussed and the yield : maturity index, based on production per day from seeding to maturity, is seen to be an aid in selection. It is recommended that crop hazards should be considered in mapping out areas for regional testing.

INTRODUCTION

The objective of many oat-breeding programs is to combine early maturity and high yield. Early maturity is required to escape damage from fall frosts, to avoid poor harvesting conditions, or to fit a given cropping plan. Since the aim of all breeding research is to maintain or increase productivity, the success of breeding for early maturity depends on the association between maturity and yield.

Because of the importance of early maturity in parts of central Alberta a preliminary study was made from several hybrids at Lacombe. A significant positive correlation was found between yield and maturity; in other words the later the maturity, the higher the yield.[3] This observation is contrary to a report by Immer and Stevenson (5) who, in studying 280 strains of oats, found that the average yield was reduced 3 bushels per acre for every day that maturity was delayed. This they attributed to greater damage from leaf rust in late-maturing strains; however, they also observed that in some seasons late-maturing varieties and strains of oats were superior in seed production. Similarly, a negative correlation between maturity and yield of 146 wheat varieties was reported by Goulden and Elders (3) who observed that early-maturing varieties had escaped damage from stem rust. Negative correlations between length of vegetative period and yield in wheat were also found by other workers (1, 6). In spite of these findings, an opinion held by many cereal workers was expressed by Clark (2) who stated that although early maturity is desirable in wheat, it is seldom considered an advantage as it is usually accompanied by reduced yield.

The present study was undertaken to examine the relationship between yield and maturity in oats with particular reference to the importance of this relationship to breeding, selection, and testing methods.

MATERIALS AND METHODS

The data used in this study were obtained from the annual reports of the Associate Committee on Plant Breeding of the National Research Council of Canada and the Canada Department of Agriculture for the years

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[3]*Unpublished data.*

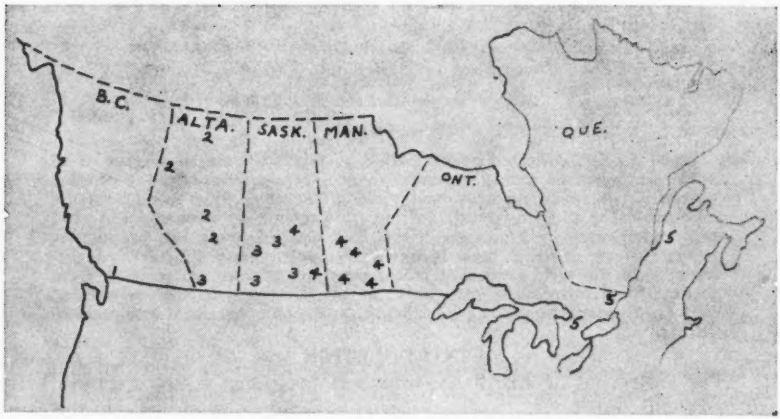

FIGURE 1. Map showing stations involved in co-operative oat tests. Approximate station locations are indicated by figures which have been coded to represent zones.

1945-1958 inclusive. These reports provided station and zonal averages for yield and maturity from co-operative tests of standard oat varieties and selections in advanced stages of testing. For the purposes of this paper, selections will also be referred to as varieties. The number of varieties in the tests ranged from 15 to 30. Three varieties were in all tests while the remainder were in tests from 1 to 3 or more years depending on their performance. For any one year, the same varieties were grown at an average of 16 stations across Canada located approximately as shown in Figure 1. Results were not obtained from all stations each year. The stations were coded to five zones (Table 1). The method of sampling ensured that a wide range of varietal material was tested simultaneously under varied environmental conditions.

From these data, correlation coefficients (r) for maturity and yield were determined for each of the five zones. A yield : maturity index calculated after the manner of Guitard *et al.* (4) was determined for each variety in each zone for the year 1955. This index represents the production in bushels per acre per day of development from seeding to maturity.

Individual plot data for maturity and yield were obtained for four stations in 1955. These data provided material for a covariance analysis (7) for which each station was treated separately.

RESULTS

Correlation coefficients for years and zones are presented in Table 2. Most were positive, supporting the view that high yield is associated with late maturity. Zone 2 coefficients were consistently positive with a mean of +.641. Zone 3 results were similar but the values were lower, the average being +.472. Negative correlations were found for some years in Zones 1, 4, and 5, and in the latter the mean coefficient was negative.

TABLE 1. — LOCATION, MAIN CROP HAZARDS, DAYS TO MATURITY, AND OAT PRODUCTION FOR FIVE ZONES OF TESTING[1]

Zone	Location	Main crop hazards	Av. time to mature days	Av. yield bu./ac.
1	S. W. British Columbia	Periodic hot dry ripening season	105	65
2	Black and grey soils of central and northern Alberta	Frost damage to late-maturing varieties	106	87
3	Brown and dark brown soils of Alberta and Saskatchewan	Drought	96	61
4	Black soils of Saskatchewan and Manitoba	Stem and crown rust, particularly at Manitoba stations	95	81
5	Co-operating stations in Ontario and Quebec	Stem and crown rust, Septoria, and excessive summer heat occasionally	89	65

[1]These zone descriptions are for the years 1948-1958 inclusive. Prior to 1948, Zones 1-5 represented provinces from British Columbia to Ontario, inclusive.

Because of obvious locational differences in the ranking of varieties for yield in Zone 4, r values were also determined for Winnipeg, Manitoba, and Melfort, Saskatchewan. The mean value for Winnipeg was similar to that of Zone 5, while the mean coefficient for Melfort resembled that of Zone 3. This diversity in ranking of varieties was not so obvious for other zones.

The widest range in r values between zones occurred in 1955 and for this reason data from this year were used to compute the yield : maturity index to evaluate the "efficiency" of varieties in the five zones (Table 3). Indexes were also calculated separately for Winnipeg and Melfort in Zone 4. It should be emphasized that there was a maturity range of only 9 days between varieties based on the means for all zones, even though for individual stations the range was as high as 13 days. The varieties in this table are arranged in order of decreasing time to maturity and coded according to maturity and zone of origin. Hence variety 1-4 is the latest maturing and originated in Zone 4, and 20-4 is the earliest and originated in the same zone. Variety 2-0 is the only exception to this arrangement, due to the fact that it is a foreign introduction.

There was a trend toward greater efficiency for late-maturing varieties in Zones 1, 2, and 3 with the strongest expression in Zone 2. The trends were in opposite directions for Winnipeg and Melfort in Zone 4 and these were masked in the indexes obtained from the zonal averages. A point of interest was that varieties which originated in Zone 4 were, with one exception (3-1), more efficient than those from other sources at Winnipeg

TABLE 2. — CORRELATION COEFFICIENTS (r) FOR YIELD AND MATURITY OF CO-OPERATIVE OAT TESTS BY ZONES FOR THE YEARS 1945-1958

Year	No. var. in test	Zone 1		Zone 2		Zone 3		Zone 4				Zone 5	
									r				
		No. of tests	r	No. of tests	r	No. of tests	r	No. of tests	Zonal av.	Winnipeg	Melfort	No. of tests	r
1945	25	–	†	3	+.157	5	+.182	4	+.371	+.388	−.001	1	+.027
1946	30	–	†	3	+.568**	4	+.657**	5	+.744**	+.567**	+.624**	1	+.453*
1947	30	–	†	4	+.753**	5	+.060	3	+.273	†	+.033	2	−.386*
1948	15	1	+.471	4	+.832**	5	+.772**	5	+.730**	+.516*	+.686**	2	+.531*
1949	25	1	+.260	1	+.576**	5	+.601**	7	+.309	+.264	+.386	2	+.054
1950	25	1	+.148	1	+.552**	5	+.062	5	+.692**	†	+.685**	2	−.075
1951	25	1	+.185	2	+.568**	4	+.178	7	+.341	−.303	+.286	2	−.290
1952	20	1	+.531*	3	+.460*	5	+.547**	6	+.391	−.304	+.482*	1	+.181
1953	16	1	−.310	2	+.529*	5	+.454	5	+.016	†	−.226	2	−.348
1954	20	–	†	–	†	4	+.184	6	−.253	−.288	−.103	2	−.328
1955	20	1	+.853**	3	+.840**	6	+.620**	5	−.086	−.612**	−.785**	2	−.749**
1956	25	1	+.752**	3	+.921**	5	+.745**	5	+.470*	−.385	+.844**	2	−.174
1957	25	1	+.874**	4	+.841**	6	+.786**	7	+.020	−.798**	+.757**	3	+.336
1958	25	1	−.295	4	+.740**	5	+.758**	6	+.585**	+.300	+.464*	3	−.045
Mean			+.347		+.641		+.472		+.329	−.060	+.407		−.058

*Significant (P = .05)
**Significant (P = .01)
†Data incomplete

TABLE 3. — YIELD: MATURITY INDEXES OF 20 VARIETIES ACCORDING TO PRODUCTION IN BUSHELS PER ACRE PER DAY OF DEVELOPMENT FROM SEEDING TO MATURITY FOR FIVE ZONES OF TESTING, 1955

Variety	Zone 1	Zone 2	Zone 3	Zone 4			Zone 5
				Zonal av.	Winnipeg	Melfort	
1-4	.67	1.00	.85	.76	.42	.90	.46
2-0	.61	.94	.78	.63	.34	.89	.50
3-1	.61	1.06	.82	.71	.53	.88	.45
4-3	.70	1.03	.84	.65	.38	1.03	.47
5-4	.56	.98	.87	.90	.60	.89	.60
6-4	.67	1.07	.88	.77	.55	.98	.51
7-3	.64	.98	.86	.72	.44	.89	.49
8-3	.59	.99	.90	.82	.63	.99	.51
9-4	.48	.85	.80	.89	.72	.81	.64
10-3	.56	.91	.84	.75	.53	.80	.52
11-4	.80	.78	.74	.84	.77	.68	.60
12-5	.81	.81	.81	.84	.64	.77	.57
13-4	.50	.76	.76	.84	.75	.74	.63
14-5	.58	.84	.73	.75	.42	.67	.62
15-4	.48	.82	.83	.81	.70	.77	.72
16-4	.51	.75	.82	.79	.76	.76	.69
17-4	.49	.81	.82	.80	.72	.78	.68
18-4	.53	.81	.79	.81	.63	.76	.67
19-4	.58	.87	.86	.88	.79	.77	.60
20-4	.52	.85	.79	.82	.74	.71	.56
Mean	.59	.90	.82	.79	.60	.82	.57
r value[1]	+.853	+.840	+.620	−.086	−.612	+.785	−.749

[1]Zone and station correlations between yield and maturity, from Table 2

but this was not true for Melfort. Early-maturing varieties had slightly higher indexes in Zone 5. In general, variation in the indexes increased as time to maturity increased. Thus the three earliest varieties had indexes ranging from .52 to .88, while those from the three latest-maturing varieties ranged from .34 to 1.06.

All of the foregoing results were obtained using the mean varietal values for yield and maturity for each zone. It has already been noted that divergent results for two stations in Zone 4, Winnipeg and Melfort, were masked by the zonal average. To determine whether the station averages themselves might mask plot differences and to assess more critically the relationship between yield and maturity, a covariance analysis was conducted using individual plot data obtained from four stations for 1955. The results are given in Table 4.

From these data it will be noted that varietal differences in yield were significant for all stations. Likewise, the varietal differences in yields adjusted for differences in maturity were significant at all stations. However, the reduction in error variance of yield due to fitting for regression was significant only at Saskatoon and Winnipeg and, at these two stations, the adjusted and unadjusted means for yield showed an appreciable difference in ranking.

TABLE 4. — INDIVIDUAL PLOT ANALYSIS OF VARIANCE AND COVARIANCE FOR TESTS AT FOUR LOCATIONS IN 1955. THE SAME 20 VARIETIES WERE IN TESTS WITH SIX REPLICATIONS.

Location	Source of variation	Mean squares and products			F	F	F	r	b
		Sx^2	Sxy	Sy^2	(1)	(2)	(3)	(4)	(5)
Lacombe	Total	14.41	32.92	215.86				+.589	2.28
	Variety	73.00	221.89	875.58	9.67**	5.31**	3.04	+.873	30.39
	Error	3.28	−2.98	90.51				−.173	−.91
Saskatoon	Total	10.57	106.82	14317.10				+.274	10.10
	Variety	30.74	−136.32	24140.32	1.93*	3.93**	38.31**	−.158	−4.43
	Error	6.74	153.02	12450.82				+.529	22.70
Winnipeg	Total	11.95	−207.42	13453.40				−.525	−17.32
	Variety	63.58	−1123.95	55455.00	10.13**	7.12**	10.34**	−.595	−17.66
	Error	2.14	−33.28	5473.15				−.300	−15.55
Ottawa	Total	11.83	−.88	41.92				−.040	−.07
	Variety	66.31	−2.89	72.05	1.99*	1.99*	.047	−.042	−.04
	Error	1.48	−.50	36.20				−.068	−.34

(1) To test the significance of difference in unadjusted means
(2) To test significance of difference in adjusted means
(3) To test significance of reduction in sums of squares due to regression

(4) $\dfrac{Sxy}{\sqrt{Sx^2 \cdot Sy^2}}$

(5) $\dfrac{Sxy}{Sx^2}$

Table 4 also gives the gross correlations and regressions together with the breakdown of these coefficients into the components derived from plot means and those derived from the within plot (error) variances and covariances. The component derived from the plot means is comparable to the figures presented in Table 2.

DISCUSSION

The correlation coefficients clearly indicate a positive association between yield and maturity. Negative correlations could have resulted from climatic conditions in Zone 1 according to Taylor,* or from disease conditions in Zone 4 where, according to the Associate Committee reports, stem and leaf rust were a problem with late-maturing varieties. According to Zillinsky,* the results in Zone 5 could have been influenced by stem rust, leaf rust, Septoria, climatic conditions, or any combination thereof. Negative coefficients were not obtained in Zones 2 and 3 where it is assumed that conditions detrimental to late-maturing varieties did not predominate during the period involved in this study.

The influence of rust epidemics on studies of this kind has been pointed out by other workers (3, 5). It is also possible that other diseases, insects, fall frost, or other hazards could influence the magnitude or sign of the zonal coefficients. The effects observed would also depend upon the varieties included in the test. Thus, a test that involved varieties susceptible to a specific disease would give variable results, depending on the presence or absence of the disease. The results for 1955 are of specific interest, in this regard, for in that year the early-maturing varieties had, in general, greater rust resistance than those of later maturity. Had this been reversed, or if all varieties had possessed the same resistance, the results for that year could have been altered considerably. Another point to be considered is the range in genetic potential for both yield and maturity of the varieties included in a test. If the variability in either or both of these traits is low in one year and high in another, the results from the two years could be markedly different.

These problems are of considerable practical importance as they have a marked bearing on the results that may be obtained from varietal testing programs. For example, stations that are now included in the same zone may, because of differences in local environment or disease problems, provide very different evaluations of the yield potential of the same varieties. This is illustrated by the comparison of results from Melfort and Winnipeg, both considered to belong to Zone 4. The analysis of covariance would appear to have a useful application for assessing more adequately true differences in genetic potential for yield. However, such methods of statistical control should be considered as secondary to the real problem which is the reassessment of the present zonal structure of varietal testing.

A relationship between maturity and yield has been established and it has also been indicated that crop hazards and environment can influence the association to the extent that it may be positive or negative. In view of

*Personal communications. 1960.

these facts it is suggested that for regional or zonal testing of oat varieties it would be advantageous to do so on the basis of crop hazards, with special reference to disease and climate. In doing this, the masking of station results in zonal averages, such as was seen in Tables 2 and 3 for Zone 4, could perhaps be avoided.

These problems are also pertinent to the development of oat breeding research programs, and perhaps cereals in general. From reference to Table 3 it will be noted that the varieties which originated in Zone 4, specifically Winnipeg, had a higher yield : maturity index at Winnipeg than varieties which originated elsewhere. That this was not true for Melfort, a station now included with Winnipeg in Zone 4, suggests some regional specificity in varietal development. This is not surprising. Varieties developed at Winnipeg should possess a greater tolerance of or resistance to specific crop hazards of that area than would varieties that originated at another location. However, the question remains as to what breeding, selection, and testing procedures to adopt in order to improve varieties from the standpoint of maturity and yield. There may be only a remote possibility of improving yield appreciably in any given maturity class. For example, referring to Zone 2 in Table 3, would it be reasonable to expect a cross between varieties 1-4 and 20-4 to provide early segregates that would yield more than 1-4 under favourable conditions? The answer to this question depends on whether or not the association between these traits is of genetic origin. If it is, then the use of mutagens or methods such as interspecific crossing may have particular application.

The yield : maturity index may be a valuable aid in making selections in tests with low experimental error. The success of this technique would depend on the range that might be expected within any maturity class and the consistency of performance of varieties from year to year. The performance of 19-4 (Table 4) offers encouragement in this respect because it has, except in Zone 5, a relatively high efficiency for its maturity class.

If the association between yield and maturity is not genetic, a shift in emphasis in oat-breeding programs would appear to be warranted. For conditions where early-maturing varieties are desired, the emphasis should be directed toward quality and agronomic features other than yield. Additional lodging resistance, for instance, would render varieties more suitable for production under heavy fertilization and thus the oat breeder could improve the yield potential under optimum conditions while maintaining the desired maturity.

From the standpoint of production, in areas where serious hazards are rarely encountered, late-maturing varieties can be expected to render greatest yields and should receive major consideration. However, in spite of the greater productivity of late-maturing varieties, early ones may serve better as a companion crop, or they may be necessary if fall-sown crops are to follow spring-sown oats on the same piece of land. For these, and perhaps other less obvious reasons, the disadvantage of productivity would be offset by advantages.

ACKNOWLEDGEMENTS

The author is grateful to J. G. Stothart, A. D. McFadden, L. P. V. Johnson and F. J. Zillinsky, for reviewing the manuscript and offering helpful suggestions; and to H. T. Fredeen, who aided in statistical interpretation and also made helpful suggestions in preparing the manuscript. He also wishes to acknowledge the use of the reports of the Associate Committee on Plant Breeding of the National Research Council of Canada and the Canada Department of Agriculture, the contents of which made this study possible.

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THE USE OF SEQUENTIAL SAMPLING IN EMBRYO TESTS FOR LOOSE SMUT IN BARLEY SEED

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[Received for publication May 23, 1961]

ABSTRACT

One hundred and forty lots of barley seed were subjected to an embryo test for the mycelium of *Ustilago nuda* (Jens.) Rostr. Ten samples of 100 embryos were examined from each lot. It was found that the use of sequential sampling, instead of single or double sampling, in making routine tests for loose smut in barley seed, would result in a considerable saving of time and effort as far as determining whether seed stocks contain more or less than the percentage of smut set as the limit of tolerance. However, if the growers require a more exact knowledge of the level of infection in their stocks of seed, it would be preferable to examine 1,000 embryos from each stock, using the single sampling method.

INTRODUCTION

Since Simmonds (8) developed the embryo test for the detection of the mycelium of loose smut fungi in embryos of cereal seeds, considerable attention has been given to its use as a routine test. One of the questions considered has been the size of sample necessary to give a reliable indication of the percentage of kernels carrying the infection in any given stock of seed. The number of embryos examined from each sample in routine tests made by different workers has varied as follows: Russell (6), average of 475; Russell and Popp (7), 600 and 500 at Saskatoon and Winnipeg, respectively; Kavanagh and Mumford (2) in Minnesota, 500; Morton, Tool and Hagen (4) in North Dakota, several hundred; Marshall (3) in Cambridge, England, 1,000; and Pedersen (5) in Denmark, 1,700. Since 1951, it has been the practice at Saskatoon to examine approximately 1,000 embryos from every sample and, in special cases, duplicate or triplicate tests have been run.

The regulations presently in force in Canada state that seed barley carrying more than 4 per cent of loose smut is not eligible for registration. Since the embryo test has been adopted as one of the criteria by which barley seed stocks are accepted or rejected for registration, it seems worth while to reconsider the practice of examining a specified number of embryos from each sample.

According to Goulden (1) use may be made of single sampling, double sampling or sequential sampling in carrying on work of this nature. However, he points out that the adoption of a plan of sequential sampling will give equally reliable results by making a substantially smaller number of observations than would be required with single or double sampling. By adopting sequential sampling in making routine embryo tests it should be possible to reduce the amount of work involved without sacrificing accuracy. This is important when a large number of samples are to be tested.

METHODS

Description of the Sequential Sampling Plan

The selection of a sequential sampling plan is based upon two considerations: the tolerance limit for loose smut infection (4 per cent), and

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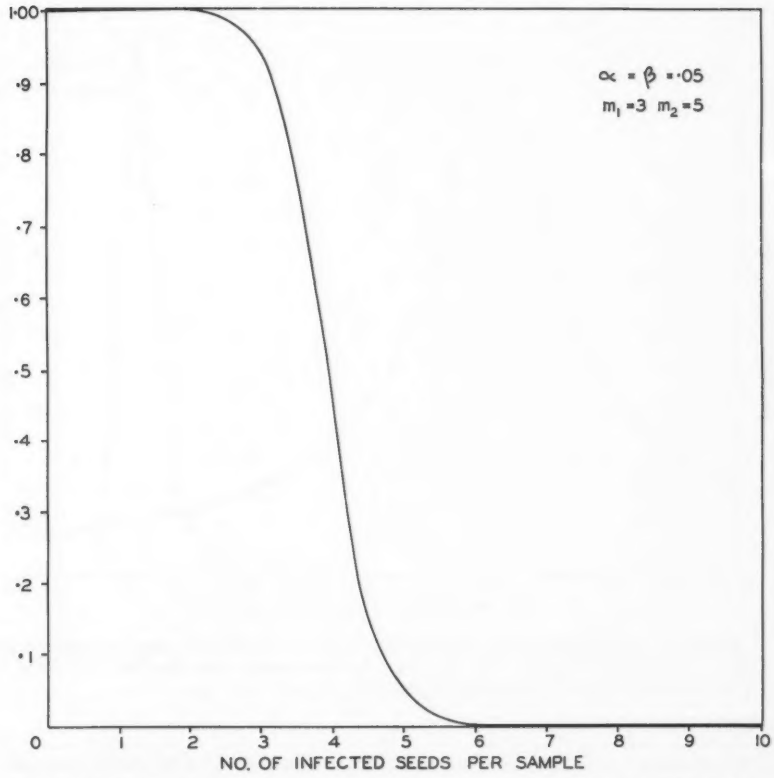

FIGURE 1. The curve shows the probability of accepting seed lots having different numbers of infected seeds, when the limit of tolerance is 4 per cent, and when the producer's and buyer's risks are 1 in 20 ($\alpha = \beta = .05$). Arbitrary levels m_1 and m_2 ($m_1 = 3$ and $m_2 = 5$) are chosen such that it is a serious matter to reject a seed lot with 3 seeds carrying smut or to accept a seed lot with 5 seeds carrying smut.

the protection afforded by the plan to both the producer and the buyer. With the present situation, it is desired to accept all seed lots containing 3 or less ($m_1 = 3$) infected seeds per hundred, and to reject all samples having 5 or more ($m_2 = 5$) per 100 seeds. Furthermore, it seems reasonable to apportion risk equally between producer and buyer, so that there would be a 1 in 20 chance of rejecting a seed stock with less than 4 per cent loose smut ($\alpha = .05$), and a 1 in 20 chance of accepting a stock with more than 4 per cent ($\beta = .05$) infection.

Having decided upon these specifications, it is then possible to calculate the probability of accepting lots having different numbers of infected seeds (assuming a Poisson distribution), and to predict the average number of samples which must be examined from these lots. These values were calculated for 14 points and are shown in Figures 1 and 2 as the Operating

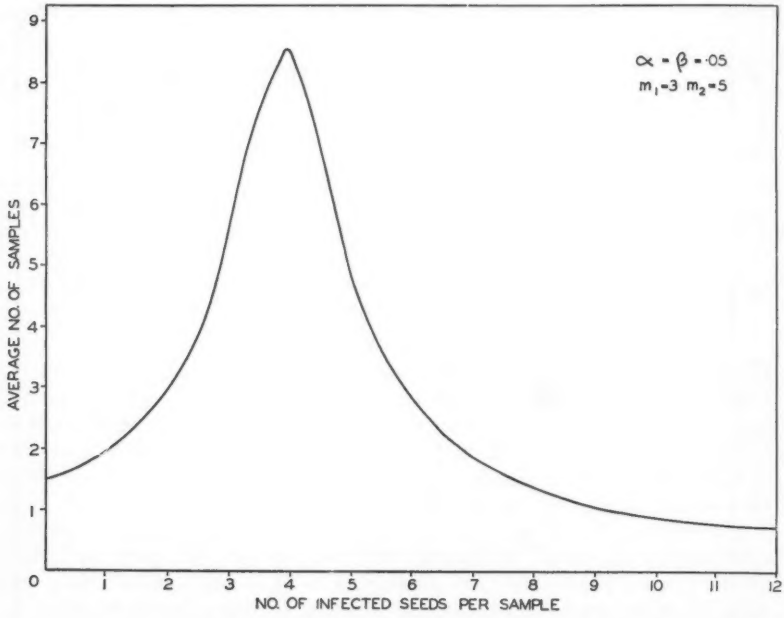

FIGURE 2. The curve shows the average number of 100-seed samples required to reach a decision to accept or reject a seed lot for loose smut, when the limit of tolerance is 4 per cent (see Figure 1 for α, β, m_1 and m_2).

Characteristic (OC) and the Average Sample Number (ASN) curves, respectively (9). These curves completely define the sequential sampling plan. A notable feature of a sequential scheme is illustrated in Figure 2, i.e., the amount of sampling required decreases very rapidly as the amount of smut infection becomes less, or more than 4 per cent, the tolerance limit specified.

In order to carry out the plan in practice, acceptance and rejection numbers were calculated for each stage of sampling, i.e., for successive samples of 100 seeds each. These values are shown in Table 1 up to 17 samples, at which stage this plan would normally terminate if no decision had already been reached. If, in the first sample of 100 embryos, 10 or more show loose smut mycelium the stock is rejected. If the number of infected embryos is 0 to 9, a second sample of 100 embryos is examined. If now the cumulative total number of infected embryos is 14 or more, the seed stock is rejected; if it is 2 or less, the stock is accepted; and if it is 3 to 13, a third sample of 100 embryos is examined. This procedure is followed with each stock until it is either accepted or rejected. If, after 17 samples have been examined, no decision has been reached, stocks having a cumulative total of 68, or less, infected embryos would be accepted and those with 69 or more would be rejected.

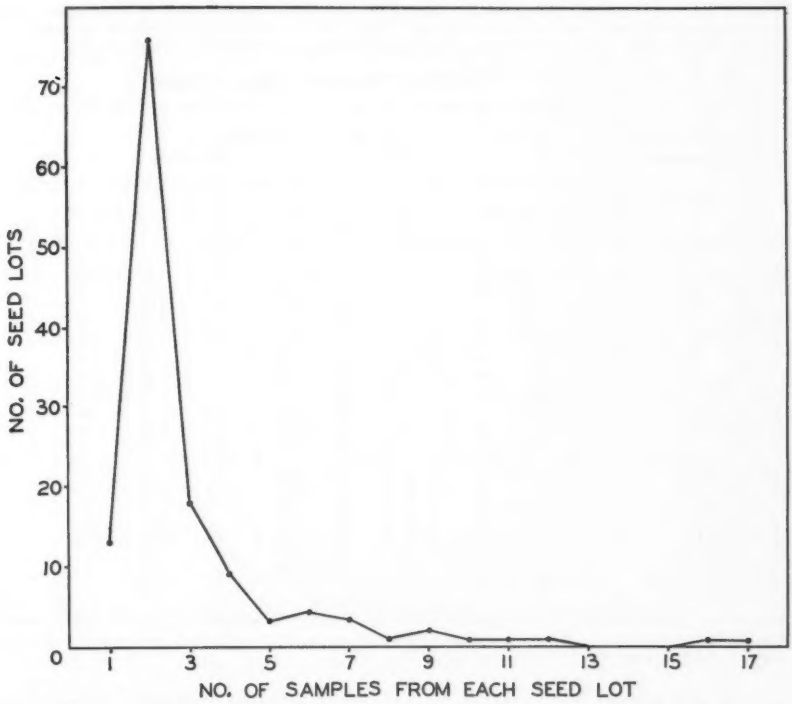

FIGURE 3. Distribution of seed lots on the basis of the number of samples required to reach a decision to accept or reject them on a sequential sampling plan for the presence of loose smut mycelium.

Test of the Plan

In order to test the efficiency of this plan, 70 stocks of barley seed from commercial growers and experimental plots were used. Two 1,000-embryo lots of each stock were examined in samples of 100 embryos each. The number of embryos infected with *Ustilago nuda* in each sample was recorded. In any sequential sampling plan, the number of samples examined depends on the number of infected embryos found in successive samples but, for purposes of this study, all of the embryos were examined.

RESULTS AND DISCUSSION

If the plan of sequential sampling, outlined in the previous section and Table 1, is applied to all the data accumulated in this investigation, but the maximum number of samples is limited to 10, the 140 lots could be accepted or rejected by examining a total of 45,100 embryos instead of 140,000. Figure 3 shows that a large majority of the seed lots would have been rejected or accepted on the first, second or third sampling. Only 21 lots would have required the examination of more than 5 samples, and only 11 would have necessitated examining the limit of 10 samples.

TABLE 1. — SEQUENTIAL SAMPLING PLAN SHOWING THE LEVELS OF ACCEPTANCE AND REJECTION OF BARLEY SEED STOCKS ON THE BASIS OF A 4 PER CENT LIMIT OF TOLERANCE

Samples of 100 embryos	Cumulative numbers of diseased embryos		
	Degrees of protection $\alpha = 0.05$ $\beta = 0.05$		
	accept	retest	reject
First	—	0 to 9	10 or more
Second	2 or less	3 " 13	14 " "
Third	5 " "	6 " 17	18 " "
Fourth	9 " "	10 " 21	22 " "
Fifth	13 " "	14 " 25	26 " "
Sixth	17 " "	18 " 29	30 " "
Seventh	21 " "	22 " 33	34 " "
Eighth	25 " "	26 " 37	38 " "
Ninth	29 " "	30 " 40	41 " "
Tenth*	33 " "	34 " 44	45 " "
Eleventh	37 " "	38 " 48	49 " "
Twelfth	41 " "	42 " 52	53 " "
Thirteenth	45 " "	46 " 56	57 " "
Fourteenth	49 " "	50 " 60	61 " "
Fifteenth	53 " "	54 " 64	65 " "
Sixteenth	56 " "	57 " 68	69 " "
Seventeenth	68 " "	—	69 " "

*When the sequential process is terminated at the tenth sample, seed lots with 40 or fewer diseased embryos are accepted and those with 41 or more are rejected.

If the percentage of infection determined by examining 2,000 embryos from each stock is taken as its true level of infection, then 136 out of 140 tests would have placed the stocks of seed in their proper categories. In four stocks, one of the lots had more than 40 infected embryos while its duplicate fell within the limit of tolerance. In the remaining tests the decision agreed with the placing according to the average of the two 1,000-embryo lots. However, in the four cases cited, the error would not have been very serious because the seed stocks from which they came were borderline cases, in which the average infection differed only slightly from the limit of tolerance. In actual practice, the ascertained risk was less than the theoretical risk in this investigation.

As a matter of interest the regular sequential sampling plan was applied to the 10 lots that were not properly placed at the end of the tenth sampling. Four of these were placed after testing from 11 to 17 samples, and in each instance the placing agreed with the arbitrary placing made at the conclusion of the tenth sampling. The proper placing of the other six lots had not been revealed at the end of the seventeenth sampling. According to arbitrary decisions made at this stage, three received the same placing that they were given at the end of the tenth sampling, and three were placed differently. These results suggest that testing more than 10 samples from any one seed lot has limited value from the practical

TABLE 2. — APPLICATION OF THE SEQUENTIAL SAMPLING PLAN TO DATA FROM DUPLICATE LOTS OF 10 DIFFERENT SEED STOCKS

Stock No.	Lot No.	No. of infected embryos in each sample of 100										Total for lot	Total for stock	Av. % infected	
		1	2	3	4	5	6	7	8	9	10			in lot	in stock
1	1	2	4	4	2	4	2	3[a]	4	5	3	33		3.3	
1	2	3	4	1	3	2[a]	4	5	5	0	1	28	61	2.8	3.05
2	3	3	1	1[a]	3	3	0	4	0	3	6	24		2.4	
2	4	1	2	2[a]	4	3	2	4	2	2	3	25	49	2.5	2.45
3	5	0	3	3	3[a]	1	0	2	2	2	3	19		1.9	
3	6	3	1	1[a]	5	3	1	2	1	2	1	20	39	2.0	1.95
4	7	0	0[a]	0	1	0	2	0	0	0	0	3		0.3	
4	8	0	0[a]	1	0	0	0	0	0	2	0	3	6	0.3	0.30
5	9	8	9[r]	8	14	19	12	11	10	20	17	128		12.8	
5	10	14[r]	7	5	9	13	10	15	19	19	24	135	263	13.5	13.15
6	11	3	6	3	5	6	3	4	5	7[r]	8	50		5.0	
6	12	5	4	4	7	5	6[r]	11	3	5	6	56	106	5.6	5.30
7	13	9	8[r]	8	9	5	4	7	6	11	9	76		7.6	
7	14	6	5	8[r]	11	9	7	14	8	7	9	84	160	8.4	8.00
8	15	0	0[a]	0	0	0	0	0	0	1	0	1		0.1	
8	16	1	0[a]	1	1	0	0	0	0	0	0	3	4	0.3	0.20
9	17	0	2[a]	2	2	2	2	2	1	2	1	16		1.6	
9	18	3	2	0[a]	3	1	1	1	3	3	3	20	36	2.0	1.80
10	19	3	4	3	4	1	1[a]	0	1	5	1	23		2.3	
10	20	5	4	2	1	3	2[a]	3	0	8	2	30	53	3.0	2.65

Note: The small "a" or "r", respectively, indicates the point at which the stock could have been accepted or rejected if the sequential plan suggested in Table 1 had been in operation.

standpoint. On the other hand, the proportion of seed stocks that would not be decided at the end of 10 samplings is so small that additional tests of these particular stocks, up to the seventeenth sample, would not entail the expenditure of much additional time and effort.

The records obtained from duplicate lots of 10 of the 70 stocks of seed studied are shown in Table 2. This is done to give an indication of the variation in number of infected embryos found in the two 1,000-embryo lots from each stock, and the variation in the numbers found in the 10 samples of 100 from any one lot. As suggested by these data, the number of samples that had to be examined in each lot tended to vary inversely with the amount by which the percentage infection of the lot differed with the limit of tolerance. Thus lots 7 and 8, which contained very little smut, and lots 9 and 10, which carried a heavy infection, required the testing of only 1 or 2 samples in order to place them properly. Lots 11 and 12, on the other hand, were closer to the limit of tolerance and required the examination of a greater number of samples.

A great majority of the barley samples, subjected to the embryo test in Saskatchewan over the past few years, carried amounts of smut infection well below or well above the 4 per cent limit of tolerance. They could have been accepted or rejected, by this plan of sequential sampling, with

the expenditure of much less time and effort in connection with the embryo tests. However, if the users of registered seed barley require a fairly exact knowledge of the percentage of loose smut infection present in their stocks of seed, this method of sampling would not be satisfactory, except for those stocks coming close to the limit of tolerance. If a grower is anxious to produce crops of barley in the field that are practically free of loose smut, he can do so by using seed that has little or no smut mycelium in its embryos. In such cases it is not sufficient to know merely that the amount of infection in a stock of seed is 4 per cent or less, one needs to know more exactly the amount present. The examination of 1,000 embryos by the single sampling method would be more advantageous for that purpose than the use of the sequential sampling method, because with it only 200 or 300 embryos would be examined and the percentage of loose smut actually present would be less accurately determined.

The amount of testing needed in such routine work as here considered could be reduced still further by accepting lots that yield no infected embryos among the first 100 examined. While such a procedure is not sanctioned in the sequential sampling plan set forth in Table 1, it could have been done safely with every one of the 53 lots that had no smutted embryos in the first sample, in this investigation. Not one of these lots carried an average smut content of over 4 per cent, and most of them carried less than 1 per cent. Moreover, none of the rejected lots had less than 1 infected embryo in any single sample. If these 53 lots had been accepted on the first count, the total number of embryos requiring examination would have been reduced by 5,300, as compared with the number required by the sequential plan used.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made of the suggestions offered by the late Cornelius Reimer, of the Statistical Research Service, Canada Department of Agriculture, concerning the biometric aspects of this investigation.

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EFFECTS OF TEMPERATURE ON YELLOWING OF FLUE-CURED TOBACCO UNDER FORCED-AIR CONDITIONS[1]

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[Received for publication April 19, 1961]

ABSTRACT

Effects of temperature during the yellowing phase of the curing process on the quality of tobacco leaves of the flue-cured type were studied in small chambers, temperature and moisture being precisely controlled. With multi-stage (conventional) schedules, whereby temperature was increased by increments of 5 and 10°F. within the range of 80 to 110°F., the leaf quality was equivalent to that obtained from a two-stage schedule, in which 90°F. was maintained for 24 hours and 105°F. for the rest of the yellowing phase. Both procedures gave only slightly higher quality than single-stage schedules in the range of 95 to 105°F. Tobacco which yellowed at temperatures outside the latter range, especially below 90°F. and above 110°F., had comparatively low quality. The temperature response was practically unaffected by variations in saturation deficit up to 75 grains of moisture per pound of dry air.

INTRODUCTION

Yellowing, colour-fixing, and mid-rib drying of tobacco leaves constitute three distinct phases of the curing process. To induce a colour change from green to yellow, the leaves are maintained in a living condition during the initial yellowing phase. Since a further colour change from yellow to brown occurs in living tissue, successively higher temperatures and lower relative humidities than those for yellowing are used in the colour-fixing phase to kill and dry the leaf tissue and so preserve the desirable yellow colour. Still higher temperatures and lower relative humidities in the mid-rib drying phase completely desiccate the mid-ribs.

Leaf quality may largely depend on the precision of manipulation of environmental conditions during the yellowing phase. For instance, a too-rapid increase in temperature or a sudden decrease in relative humidity produces undesirable green grades; prolonged high humidity produces undesirable dark grades. Middleburg (3), using micro-kilns, in which variations in environmental conditions were minimized in comparison with standard-sized kilns, obtained good quality leaf by maintaining a constant wet bulb temperature of 90 to 100°F. The drying capacity of the air was slowly increased during the yellowing phase by raising the dry bulb temperature. This procedure differs little from the conventional method used in yellowing Canadian tobacco, where a gradual increase in temperature from 80 to to 110°F. coincides with a gradual decrease in relative humidity from 100 to 60 per cent. However, because of the inadequacy of facilities for precise control within the ranges indicated above, information has been lacking on the effects of variations in temperature or moisture on the quality of Canadian flue-cured tobacco. Small chambers, conditioned with forced air, provide the precise control required for such studies. Previous investigations[2] showed that the leaf quality obtained in kilns equipped with a forced-air system was equivalent to that obtained with a natural convection (conventional) system.

[1]Contribution No. 23, Research Station, Research Branch, Canada Department of Agriculture, Harrow, Ont.

[2]Walker, E. K., and J. Williams. Tobacco curing investigations in 1955. Research rept., Ontario Research Foundation, Toronto, Ont. *Unpublished.*

TABLE 1. — LIST OF THE YELLOWING CONDITIONS (TREATMENTS) USED IN FIVE EXPERIMENTS. A = TEMPERATURE (°F.); B = SATURATION DEFICIT (GRAINS MOISTURE PER POUND DRY AIR); C = DURATIONS OF EXPOSURE IN TWO- AND MULTI-STAGE SCHEDULES

Treatment	Experiment											
	1		2		3		4			5		
	A	B	A	B	A	B	A	B	C	A	B	C
1	80	25	85	25	85	75	90	25		90 95	25 75	24 hours Remainder of phase
2	88	25	90	25	90	75	95	25		90 95	25 25	24 hours Remainder of phase
3	96	25	95	25	95	75	100	25		90 100	25 75	24 hours Remainder of phase
4	104	25	100	25	100	75	105	25		90 100	25 25	24 hours Remainder of phase
5	112	25	105	25	105	75	80 90 95 105 110	20 37 48 60 80	Until yellowing initiated Until yellowing well advanced Until lamina yellowed Until veins yellow Until mid-ribs yellow	90 105	25 75	24 hours Remainder of phase
6	120	25	110	25	110	75	80 90 95 105 110	10 10 10 10 10	Until yellowing initiated Until yellowing well advanced Until lamina yellowed Until veins yellow Until mid-ribs yellow	90 105	25 25	24 hours Remainder of phase

With precision equipment available, the present study was undertaken to determine the effects of temperature on the quality of tobacco during the yellowing phase of the curing process.

MATERIALS AND METHODS

The curing studies were conducted in well-insulated, forced-air chambers, 106 cubic feet in volume. Temperature and moisture were regulated by means of Aminco-Aire[1] units specifically designed for the curing of tobacco. Moisture in the air was measured as saturation deficit rather than as relative humidity because the drying capacity of air is unaltered by temperature at constant saturation deficit.

The treatments in five experiments are shown in Table 1. There was a single cure (priming 1) for Experiment 1, four cures (primings 2 to 5) for each of Experiments 2 and 3, and five cures (primings 1 to 5) for each of Experiments 4 and 5. The primings consisted of leaves at all stalk positions from the lowermost sand leaves in priming 1 to the uppermost tip leaves in priming 5. Experiments 1 to 3 were conducted in 1958, and Experiments 4 and 5 in 1959.

Saturation deficit was varied among experiments in 1958 and between treatments in 1959 to evaluate its possible interdependence with temperature thereby assuring a proper evaluation of temperature effect.

Five laths, supporting leaves selected for uniformity of size, maturity and freedom from leaf disorders, were placed in each of six chambers per cure. Leaf samples were collected at the end of the yellowing phase from one lath in each chamber, quick-frozen in sealed plastic bags, and placed in cold storage for subsequent pigment analysis (5). The remaining four laths in each chamber were transferred to a drying chamber for completion of the curing process. The latter laths served as the treatment replications for the single cure in Experiment 1, whereas the cures, or the averages for the four laths, served as replications in Experiments 2 to 5.

Representative samples of the cured tobacco were dried and then ground in a Wiley mill with a 3-millimetre screen for photoelectric measurements of colour and brightness. The portion of each ground sample collected between 20 and 28 Tyler No. sieves, after shaking for 3 minutes in a Ro-Tap shaker, was equilibrated for 1 week at 80°F. and a relative humidity of 60 per cent. Lots of tobacco representing the choice and fine quality grades by the 1958 grading system were similarly prepared for colour and brightness measurement, to obtain ideal values for comparison with the sample values in each year. The tri-stimulus colour measurements of "L" (visual lightness), "a" (red-green), and "b" (yellow-blue) were obtained on a Gardner automatic colour meter[2], the positive values signifying the predominance of visual lightness, red and yellow, and negative values of visual darkness, green and blue. In order to obtain individual readings of colour difference between sample and ideal values, the tri-stimulus measurements were composited by means of the formula

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where ΔE denotes total colour difference, and ΔL, Δa and Δb the respective

[1]American Instrument Co., Inc., Silver Spring, Md.
[2]Gardner Laboratory Inc., Bethesda, Md.

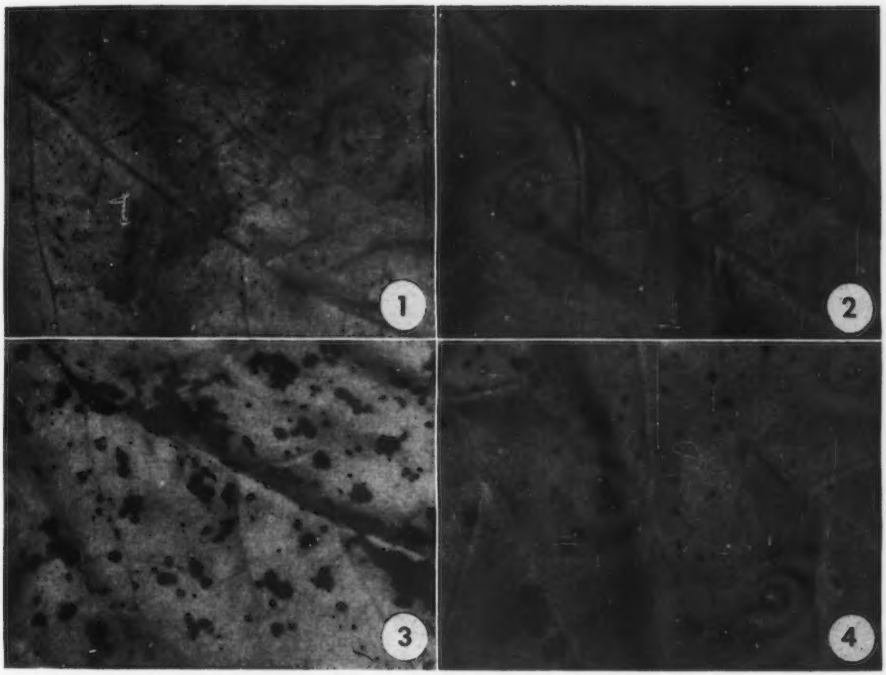

FIGURE 1. Response of tobacco leaves to changes in the yellowing environment. *No. 1*, typical concentration of fleck-like spots in leaves yellowed at 85°F. and 75 grains; *No. 2*, clear appearance of leaves yellowed at 105°F. and 75 grains; *No. 3*, typical concentration of splotching in affected leaves yellowed at 110°F. and 25 grains; *No. 4*, typical concentration of splotching in affected leaves yellowed at 110°F. and 75 grains.

TABLE 2. — GRADE INDICES (CENTS PER POUND) OF TOBACCO YELLOWED BY VARIOUS SCHEDULES IN FIVE EXPERIMENTS

Treatment	Experiment				
	1	2	3	4	5
1	32.2	46.8	46.6	55.5	55.5
2	29.0	50.6	48.8	55.9	55.6
3	51.4	51.0	51.2	54.0	54.6
4	51.8	51.4	51.6	54.7	56.2
5	43.2	52.8	50.8	56.9	57.2
6	1.0	52.8	52.0	56.7	56.6
L.S.D. .05	3.7	3.2	3.4	2.0	1.5

colour differences between sample and ideal values. The brightness measurements, which were obtained on an Agtron Model F meter[1], are readings between the Agtron standards 5010.5 as 0 and 5040 as 100.

The system of leaf grading in 1958 differed from that in 1959. The method used experimentally since 1938 was discontinued in 1959 in favour of the official grading system of the Ontario Farm Product Grades and Sales Act, which includes several additional classifications of leaf.

The significance of grade index, colour, brightness and pigment values was tested by an analysis of variance.

RESULTS

Effects on Leaf Quality

Table 2 gives the average grade indices for the various treatments in each experiment. The grade index values are the weighted average market prices for the grades of leaf for each treatment. In Experiments 1 to 3 tobacco yellowed below 90 and above 110°F. had relatively poor quality, and the difference in saturation deficit between Experiments 2 and 3 had practically no effect on quality. Although the treatments caused only small differences in Experiment 4, the grade index values for the single-stage schedules were slightly lower than those for the multi-stage schedules. The differences in saturation deficit (treatments 5 and 6) had little effect on leaf quality. The treatments in Experiment 5 also caused small differences, but the grade index was somewhat higher for a final yellowing temperature of 105°F. than for 95 or 100°F.

The treatments had a variable effect on the amount of undesirable dark and green pigmentation. The percentage of leaf area accounted for as small fleck-like spots decreased with rise of yellowing temperature, but was unaffected by level of saturation deficit. Splotching, a leaf condition characterized by relatively large, dark, irregularly-shaped spots, was more extensive at 25 than at 75 grains saturation deficit, and occurred only at temperatures above 105°F. However, this disorder occurred on less than 10 per cent of the leaves in any treatment. Figure 1 illustrates treatment effects on leaves in Experiments 2 and 3.

[1]Agtron Inc., Portland, Oregon.

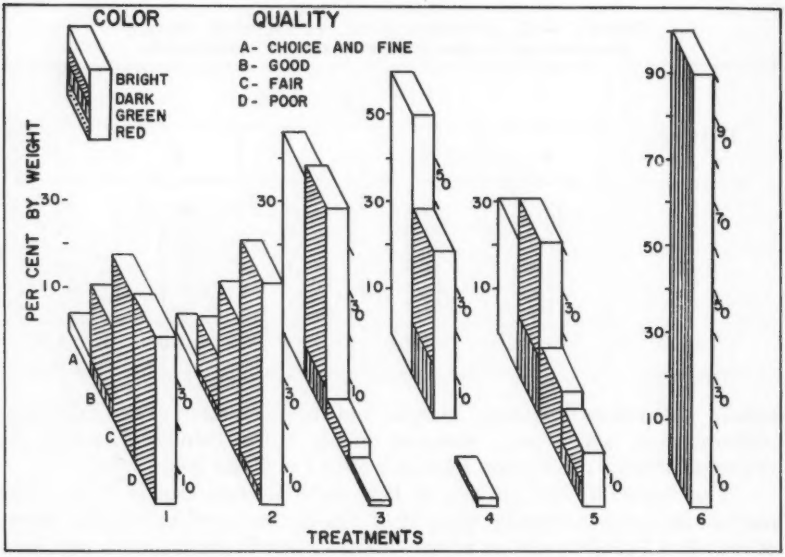

FIGURE 2. Major colour and quality classes of tobacco yellowed by means of single-stage schedules from 80 to 120°F. (Experiment 1).

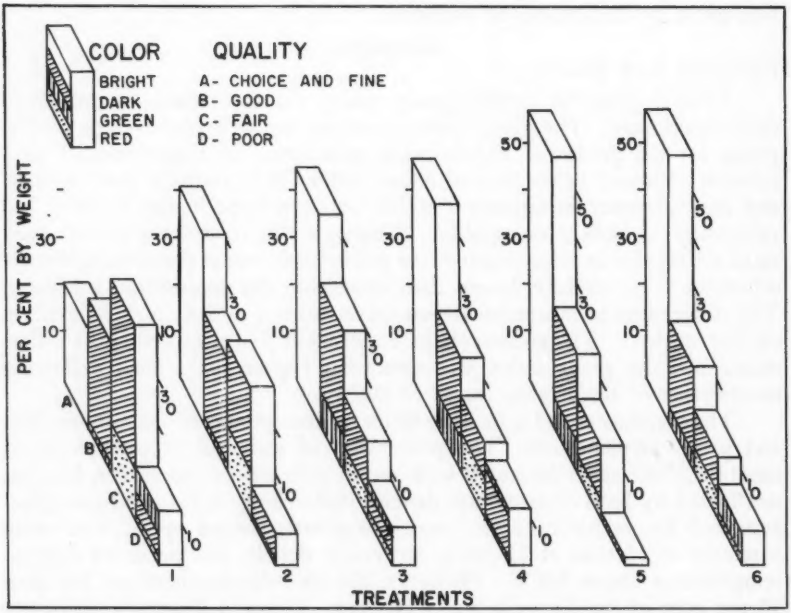

FIGURE 3. Major colour and quality classes of tobacco yellowed by means of single-stage schedules from 85 to 110°F. and a saturation deficit of 25 grains (Experiment 2).

TABLE 3. — SAMPLE AND IDEAL VALUES OF TRI-STIMULUS COLOUR (L, a AND b) AND TOTAL COLOUR DIFFERENCES ($\triangle$E) BETWEEN SAMPLE AND IDEAL VALUES OF TOBACCO YELLOWED BY VARIOUS SCHEDULES IN THREE EXPERIMENTS

Treatment	L			a			b			$\triangle$E		
	Experiment			Experiment			Experiment			Experiment		
	1	4	5	1	4	5	1	4	5	1	4	5
1	55.3	54.6	54.6	4.0	5.1	4.9	27.2	23.6	23.9	7.3	8.5	8.3
2	55.9	55.2	56.0	3.7	4.7	5.2	26.7	24.0	24.7	7.0	7.8	6.7
3	60.5	57.3	56.1	4.2	4.2	4.8	29.9	24.8	24.5	1.6	5.9	6.8
4	61.9	57.1	55.7	4.4	4.7	4.8	30.7	25.2	24.6	0.8	5.7	7.0
5	60.8	56.5	55.6	1.7	4.6	4.6	29.5	24.9	24.6	2.8	6.3	7.1
6	41.8	56.9	57.4	−0.7	4.7	5.1	18.0	24.8	25.6	24.1	6.1	5.2
7[1]	62.1	60.4	60.4	4.1	4.9	4.9	30.0	29.8	29.8			
L.S.D. .05	0.3	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.5	0.2	0.3	0.3

[1]Ideal values: average readings of choice and fine-quality grades

Figures 2 to 6 show the percentage by weight of each group of grades having similar colour and quality characteristics. Consecutively, the illustrations represent tobacco in Experiments 1 to 5.

The percentage by weight of green grades in Experiment 1 (Figure 2) increased with temperature. At the upper temperature limit of 120°F., all tobacco was classified into low-quality green grades. Temperatures of 96 and 104°F. produced maximum amounts of desirable bright and minimum amounts of undesirable dark grades.

In Experiments 2 and 3 (Figures 3 and 4), the percentage of bright grades increased and that of dark grades decreased with rise of temperature from 85 to 110°F.; the percentage of green grades was practically unaffected by temperature. The saturation deficit of 25 grains in Experiment 2 yielded slightly less of the green and slightly more of the bright grades than the saturation deficit of 75 grains in Experiment 3.

In Experiment 4 (Figure 5), the maximum percentage of choice and fine quality grades was obtained from the schedule in which temperature was increased from 80 to 110°F. and saturation deficit from 20 to 80 grains. This schedule and the constant-temperature schedules of 90 and 95°F. gave lower percentages of green grades than the constant-temperature schedules of 100 and 105°F.

A final yellowing temperature of 105°F. in Experiment 5 (Figure 6) produced more of the bright and less of the dark grades than final yellowing temperatures of 95 or 100°F.

Effects on Colour and Brightness

Tri-stimulus colour measurements for Experiments 1, 4 and 5, and brightness measurements for Experiments 1 to 3 are shown in Tables 3 and

TABLE 4. — BRIGHTNESS VALUES OF TOBACCO YELLOWED BY VARIOUS SCHEDULES IN THREE EXPERIMENTS

Treatment	Experiment		
	1	2	3
1	55.9	50.7	52.7
2	57.4	54.5	53.6
3	76.4	58.8	56.3
4	81.4	60.4	55.4
5	78.4	64.9	56.6
6	10.5	63.8	60.7
7[1]	76.1	66.9	66.9
L.S.D. .05	5.6	3.1	4.3
r with L value	+0.999**		

[1]Ideal values: average readings of choice and fine quality grades
**Significant at P = .01

TABLE 5. — TOTAL CHLOROPHYLL (A) AND TOTAL CAROTENOID (B) CONTENTS OF TOBACCO YELLOWED BY VARIOUS SCHEDULES IN FIVE EXPERIMENTS (MEAN VALUES EXPRESSED AS MICROGRAMS PER GRAM OF OVEN-DRY LEAF)

Treatment	Experiment									
	1		2		3		4		5	
	A	B	A	B	A	B	A	B	A	B
1	73	238	84	271	101	314	80	245	97	271
2	68	258	107	291	88	261	71	252	126	281
3	59	268	186	328	128	285	102	233	109	263
4	254	272	147	346	163	332	86	309	116	303
5	313	498	143	405	116	331	74	209	124	261
6	950	526	270	420	151	344	94	235	106	328
L.S.D. .05	29	25	95	80	N.S.	79	N.S.	N.S.	N.S.	N.S.

4, respectively. Tobacco yellowed at 96 and 104°F. in Experiment 1, at 100 and 105°F. in Experiment 4, and at the final yellowing conditions of 25 grains and 105°F. in Experiment 5, more closely approached the ideal colour than tobacco from other treatments in the respective experiments. In Experiment 1, the brightness values for 80, 88 and 120°F. were much lower than for 96, 104 and 112°F.; in Experiments 2 and 3, brightness increased with increase of temperature from 85 to 110°F. The increase of saturation deficit from 25 grains in Experiment 2 to 75 grains in Experiment 3 caused a slight decrease in brightness. There was a close association between the L and the brightness measurements.

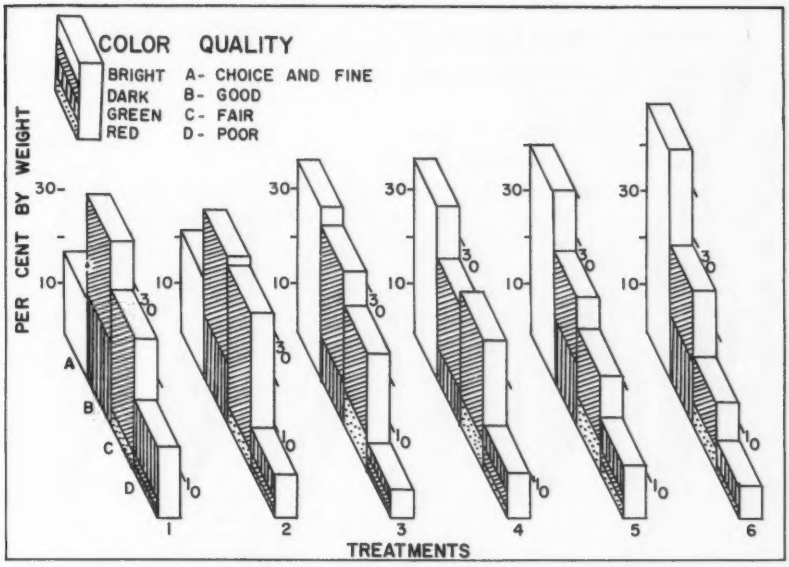

FIGURE 4. Major colour and quality classes of tobacco yellowed by means of single-stage schedules from 85 to 110°F. and a saturation deficit of 75 grains (Experiment 3).

Effects on Plastid Pigment Concentration

The average amounts of total chlorophyll and total carotenoids at the end of the yellowing phase in each experiment are given in Table 5. Both pigments increased with successive increments of temperature from 96 to 120°F. in Experiment 1; the levels of each were closely the same within the range of 80 to 96°F. Differences in concentration within the narrower range 85 to 110°F. in Experiments 2 and 3 were relatively small, although there tended to be a slight increase of both pigments up to maximal amounts at 110°F. The increase of saturation deficit from 25 grains in Experiment 2 to 75 grains in Experiment 3 appeared to have little effect on pigment concentration. Treatment differences in Experiments 4 and 5 were small and inconsistent.

Temperature and Grade Correlations

Values of r for temperature and grade index with measurements of colour, brightness and pigments in Experiments 1 to 3 are shown in Table 6. Grade index was poorly associated with each of the above measurements in Experiments 4 and 5; use of multi- and two-stage schedules prohibited correlations with temperature. In Experiments 1 to 3, temperature was positively correlated with brightness and grade index within the range 80 to 110°F.; a marked decrease in grade index and brightness above 110°F. resulted in poor associations of temperature with brightness and grade index within the range 80 to 120°F. There tended to be positive associations of

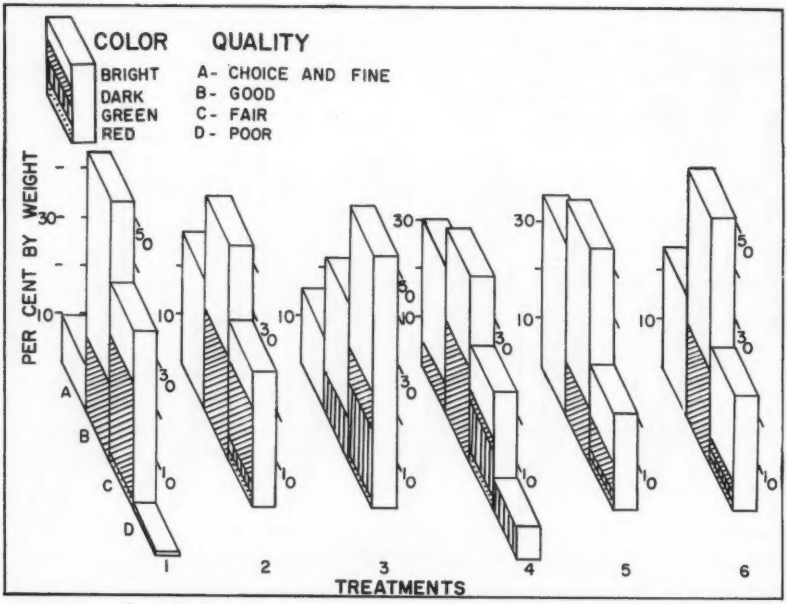

FIGURE 5. A comparison of various single- and multi-stage schedules with respect to major colour and quality classes of tobacco (Experiment 4).

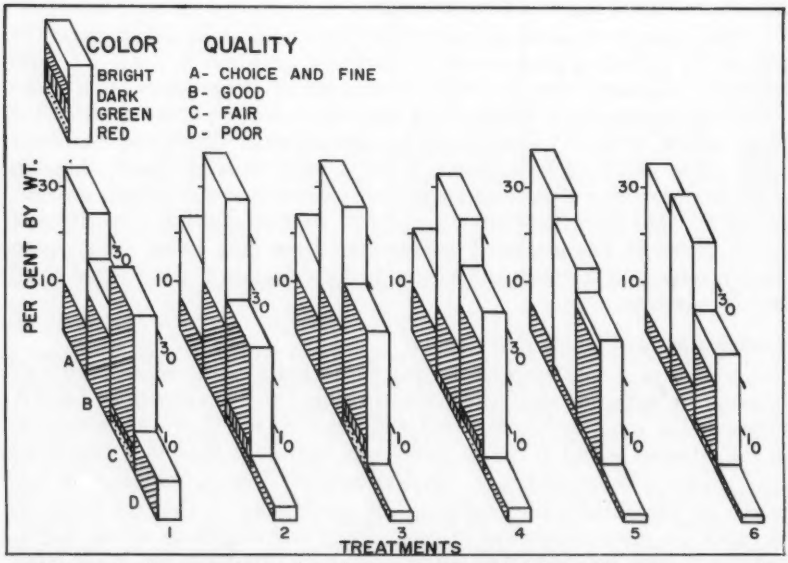

FIGURE 6. A comparison of various two-stage schedules with respect to major colour and quality classes of tobacco (Experiment 5).

TABLE 6. — CORRELATION COEFFICIENTS BETWEEN PAIRED COMBINATIONS OF DATA FROM THE 1958 EXPERIMENTS

Experiment	Variable	r	
		With temperature	With grade index
1	Temperature	—	−.317
	Grade index	−.317	—
	Total chlorophyll	+.807	−.785
	Total carotenoids	+.861*	−.524
	Brightness	−.319	+.977**
	L	−.367	+.796
	a	−.779	+.981**
	b	−.420	+.981**
2	Temperature	—	+.897*
	Grade index	+.897*	—
	Total chlorophyll	+.810	+.682
	Total carotenoids	+.987**	+.832*
	Brightness	+.963**	+.938**
3	Temperature	—	+.863*
	Grade index	+.863*	—
	Total chlorophyll	+.667	+.767
	Total carotenoids	+.670	+.404
	Brightness	+.914*	+.805

*Significant at P = .05
**Significant at P = .01

plastid pigments with temperature in each experiment, but, as with temperature, pigments were correlated with grade index only within the temperature range 80 to 110°F. The colour and brightness measurements were positively related to grade index in the 1958 experiments.

DISCUSSION

This study indicates that a single-stage temperature schedule should be in the range 95 to 105°F. Temperatures below 95°F. were unsuitable for yellowing because of fleck-like spots, whereas those above 105°F. were unsatisfactory because of splotching and green fixation. This differs from the findings of Cooper *et al.* (2), who reported that temperatures up to 115°F. were suitable for yellowing.

The results substantiate the observation by Moss and Teter (4) that green grades result from increasing temperature too rapidly in the yellowing phase; however in this study the production of such grades reached unsatisfactory levels only at temperatures above 105°F. Their observation may be valid for temperatures below 105°F. under poorly-controlled conditions in conventional kilns, where green fixation may result from a decrease in relative humidity with rise of temperature rather than from a temperature increase *per se*. The results also support the observation by Moss and Teter (4) that splotching increases with relative humidity; however, temperature level was the primary determinant as no splotching occurred below 105°F. At 120°F. an almost complete destruction of leaf tissue was manifested by rapid

fixation of a mixture of green and brown pigmentation. In contrast, splotching, which caused much less severe damage, consisted of dark spots or patches on leaves yellowed between 105 and 120°F. Since splotching occurred below the killing temperature of 120°F., it may possibly be a manifestation of relatively slow tissue destruction. Periodic examination of leaves during the yellowing phase revealed that the small fleck-like spots, which were particularly prevalent at low yellowing temperatures, remained green longer than surrounding normal tissue. This indicated that such areas were immature, and, since the incidence of the flecks was reduced by an increase of temperature, it was concluded that yellowing had been inhibited by low temperatures. However, the over-all rate of yellowing, as measured by leaf chlorophyll concentration, appeared to be closely the same for temperatures from 80 to 105°F. Biochemical reactions may have accounted for the eventual dark colour of the flecks. For instance, Baebler (1) observed that the oxidation of pyroll in the presence of chlorogenic acid and polyphenoloxidase contributed to the formation of dark pigmentation under yellowing conditions where chlorophyll was insufficiently eliminated.

The response to temperature was practically unaffected by the variations in saturation deficit; therefore, saturation deficit may be important only at levels conducive to extensive leaf drying. A slight increase in percentage of green grades at 75, as compared with 25 grains saturation deficit, indicates that the quality-limiting level may be close to 75 grains. Although the saturation deficit was varied only to supplement the main study of temperature effect, it is noteworthy that the relative humidities of 73, 77 and 81 per cent for a saturation deficit of 75 grains at 95, 100 and 105°F., respectively, approximate 75 per cent, the lower limit recommended by Cooper *et al.* (2) for yellowing.

ACKNOWLEDGEMENTS

The authors are indebted to W. Kalbfleisch, Chief, Engineering Research Service, Research Branch, Ottawa, for assistance in selection of the curing equipment used in this investigation. Appreciation is also extended to the Imperial Tobacco Company of Canada, Ltd., Montreal, Que., for advice on measurement techniques for and use of a Gardner colour meter, and to Elizabeth Back, Tobacco Substation, Delhi, Ont., for technical assistance.

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EFFECTS OF INOCULATING WHEAT SEEDLINGS WITH *HELMINTHOSPORIUM SATIVUM* AND SPREAD OF THE FUNGUS TO AN EXTERNAL SUBSTRATE[1]

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[Received for publication June 6, 1961]

ABSTRACT

Studies were conducted with wheat seedlings inoculated with *Helminthosporium sativum* and observations were made on the spread of this fungus from lesions or other sources into an external substrate. Infections caused severe injury as measured by leaf chlorosis. Injury was reduced when the seedlings were supplied with potassium nitrate solution in place of water. There was greater injury and more extensive colonization of the fungus when the nutrient solution was enriched by adding sucrose. The fungus was observed to spread readily from lesions and other sources into the adjacent environment. Soil masses around a root lesion did not inhibit the spread. The results of these studies are discussed in relation to root diseases under field conditions.

INTRODUCTION

In a recent paper (3) the general background of nitrogen nutrition relationships in studies on common rootrot of wheat was given and the pertinent literature reviewed. Primary attention was directed to studies of infections with *Helminthosporium sativum* Pamm., King and Bakke on detached wheat leaves. In this report infections of wheat seedlings will be considered, with observations on lesion formation and development of the pathogen in the crown zone. Studies on the influence of infection on the adult plant will be reported later.

The pathogen employed in this study will be referred to as *Helminthosporium sativum*, a name familiar to pathologists. Its taxonomic position, at present, may be stated as follows: *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur, whose conidial stage is *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker (*Helminthosporium sativum* P.K. and B.).

METHODS

Seedlings used in the infection studies were grown in culture tubes. This technique, described in earlier reports (3, 4), was modified to the extent of placing a small pad of absorbent cotton over the seed to maintain good moisture conditions. The seedlings were inoculated when in the second- or third-leaf stage by rubbing dry spores of *H. sativum* along the coleoptile area. A reliably virulent isolate designated H5 was used. After inoculation the cotton pad was placed over the coleoptile area. The seedlings were grown in water and in 0.5 per cent potassium nitrate solution. For some tests 2 or 3 millilitres of a 3 per cent sucrose solution were added to the cotton pad once or twice through the test period. (This, of course, would diffuse through the approximately 18 cubic centimetres in the tube.) The sucrose encouraged contaminations, both fungal and bacterial. The seedlings were grown under greenhouse conditions with some supplementary incandescent light. The seeds were not surface-sterilized, but conspicuously abnormal or diseased seedlings were eliminated when inoculations were made. Final notes were taken in from 7 to 10 days after inoculation when

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most seedlings were at the fourth-leaf stage. The amount of chlorosis was rated as 1 if an entire leaf was involved, 0.5 for half a leaf, and so on. Leaf loss was equated with chlorosis. When dry weights were taken the seedlings were removed from the tubes and dried at room temperature. Mindum wheat was mainly used because it manifests a distinct chlorosis when nitrogen is deficient. From 10 to 25 seedlings were used for each treatment in all experiments.

RESULTS

Chlorosis of Seedlings

Many experiments were conducted comparing inoculated and uninoculated seedlings with respect to the extent of leaf chlorosis. Summary data from some of these tests are shown in Table 1.

These data show a marked loss of leaf function, through chlorosis, to the extent of about one leaf for each seedling with a severe lesion.

TABLE 1. — LESIONS AND LEAF LOSS (CHLOROSIS) IN SEEDLINGS OF WHEAT VARIETIES INOCULATED WITH *H. SATIVUM*, AS COMPARED WITH UNINOCULATED SEEDLINGS

Variety	Treatment	Number of seedlings						
		Severe lesions	Healthy	Leaf loss of[1]				
				1	0.5	0.25	0	Av.
Mindum	Inoculated	127	0	95	31	1	0	0.87
,,	Check	0	60	2	9	4	45	0.12
Golden Ball	Inoculated	38	0	20	6	6	6	0.64
,,	Check	0	28	3	1	6	18	0.18
Garnet	Inoculated	24	0	20	4	0	0	0.92
,,	Check	0	18	5	13	0	0	0.64
Reward	Inoculated	23	0	19	4	0	0	0.91
,,	Check	0	20	8	12	0	0	0.70

[1]The amount of leaf loss was rated as "1" if an entire leaf was chlorotic, "0.5" for half a leaf, etc.

TABLE 2. — LESIONS, LEAF LOSS (CHLOROSIS) AND DRY WEIGHT OF MINDUM WHEAT SEEDLINGS INOCULATED WITH *H. SATIVUM*, AND UNINOCULATED; GROWN IN WATER AND POTASSIUM NITRATE SOLUTIONS

Treatment	Nutrient	Number of seedlings							Av. dry wt., gm.
		Severe lesions	Healthy	Leaf loss of[1]					
				1	0.5	0.25	0	Av.	
Inoculated	Water	37	0	25	6	0	6	0.76	0.028
Check	,,	0	31	2	7	0	22	0.18	0.031
Inoculated	KNO_3 0.5%	39	0	8	4	0	27	0.26	0.056
Check	,,	0	30	0	0	0	30	0.00	0.054

[1]The amount of leaf loss was rated as "1" if an entire leaf was chlorotic, "0.5" for half a leaf, etc.

After the characteristics of the infection reactions were established in experiments with seedlings in water, studies were conducted with 0.5 per cent potassium nitrate solution. Preliminary tests indicated a similar reaction when calcium nitrate was used, but ammonium nitrate was toxic. Potassium nitrate was selected for most tests because it is readily found in wheat tissues, as shown by Eckerson (1). Several tests with potassium nitrate were made with various modifications. The combined results of two experiments are given in Table 2.

The results show clearly the pronounced chlorosis and leaf loss in the water cultures in comparison with a much smaller loss in the potassium nitrate cultures (Figure 1). The limited data on dry weights did not show marked differences. This would appear to indicate that, through this stage of growth at least, the infection primarily affects the nitrogen metabolism and results in chlorosis.

The general trend was the same in all experiments with the potassium nitrate solution, including one in which the variety Marquis was used instead of Mindum.

Lesion Characteristics

Infected seedlings grown in culture tubes provided good specimens for observations on the extent and depth of the lesions. Seedlings were examined from 7 to 10 days after inoculation. The lesions extended about 1 to 1½ inches up the base from the seminal node. They were dark brown to almost black. The coloration was deeper than is usually seen in field specimens and deepest on the seedlings grown on potassium nitrate solution. The general appearance and outline of the lesions was much the same for seedlings growing in water or potassium nitrate. Dissection revealed that the coleoptile was completely invaded in both lots. Those in water showed extensive colonization and discoloration on the first- and second-leaf tissues beneath the coleoptile. For the seedlings in potassium nitrate solution the lesion did not extend below the first-leaf tissues in the specimens examined. The seminal node showed general discoloration with lesions extending about an inch along the roots. Dissection showed that these root lesions were confined to the cortex of the seedlings both in water and in potassium nitrate solution. When the lesions were dried and then incubated, *H. sativum* sporulations soon appeared, but somewhat more readily on those from the water-supported seedlings.

Observations were also made on lesions on pieces of detached leaves. Large, uniformly green upper leaves, from Reward wheat grown in crocks in the greenhouse, were used. Small pieces to fit into a Petri dish were inoculated in one spot with a mass of conidia, and the leaf turned over to assure contact with the moist filter paper. Germination of the spores was good.

The pieces maintained on water soon showed signs of an incipient lesion and in 2 days its diameter was about 1 inch; surrounding it was a slight but distinct halo. There was good aerial growth of hyphae but no sporulation.

In 4 days the general picture was much the same, but sporulation had commenced. Chlorosis had increased but the focal point of the inoculation retained much of its green colour. Pieces maintained on 3 per cent sucrose in place of water reacted much the same as those on water, except there was greater sporulation. There was general retardation of lesion formation in the case of leaf pieces maintained on 3 per cent potassium nitrate. The lesions were small, about half the size of those on water, with very little halo effect or chlorosis, and the dark green colour of the tissue was retained almost completely to the end of the 5-day period. It would appear that, when there is an excess of nitrogen, the colonization of *H. sativum* in leaf tissues is greatly retarded.

Influence of an Enriched External Substrate

The above experiments and observations indicate that infection interferes with nitrogen metabolism, as shown especially by chlorosis, and this can be corrected by additions of nitrogen in the form of potassium nitrate. It is thought the colonization of the fungus or the formation of a lesion drains nitrogen from adjacent tissues (3). Consequently, the amount of drainage would be limited by the size and activity of the colonization in any tissue substrate. However, if under certain conditions the fungus can extend its growth into adjacent substances, especially a substrate rich in carbon, then with this larger colonization there would follow a greater drainage. Some inoculation tests, similar in design to the above-mentioned ones, were conducted in which sucrose was used to enrich the external substrate. In most, including the one detailed here, the seedlings were supported by a 1 per cent KNO_3 solution. Mindum, Thatcher, Red Bobs and Garnet wheats were used. Six experiments were conducted and showed the same trend, namely, a greater leaf loss and, in most cases, a slight reduction in dry weight when the external substrate was enriched. The data on one test with Garnet are shown in Table 3.

Observations revealed the external ramifications of the hyphae of the pathogen in the blotter and cotton pad near the lesion, especially where sucrose had been added.

TABLE 3. — LESIONS, LEAF LOSS (CHLOROSIS) AND DRY WEIGHT OF GARNET WHEAT SEEDLINGS INOCULATED WITH *H. SATIVUM*, AND UNINOCULATED; GROWN IN POTASSIUM NITRATE SOLUTION, WITH AND WITHOUT SUCROSE

Treatment	Nutrient	Number of seedlings			Av. dry wt., gm.
		Severe lesions	Healthy	Chlorotic leaves	
Inoculated	KNO_3 + sucrose	10	0	9	0.050
Inoculated	KNO_3	10	0	6	0.055
Check	KNO_3 + sucrose	0	9	tr	0.055
Check	KNO_3	0	7	0	0.066

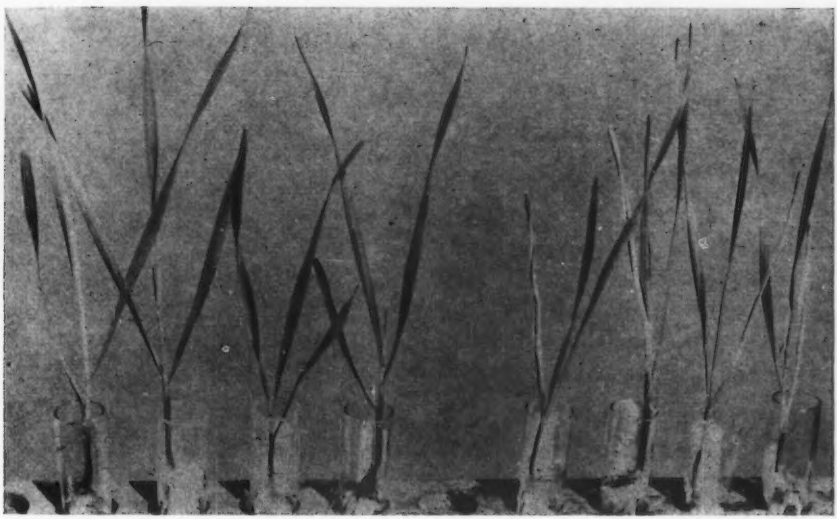

FIGURE 1. Selected seedlings of wheat inoculated with *H. sativum*, all with severe lesions; those on the *left* were supported by a weak KNO_3 solution, those on *right* by water.

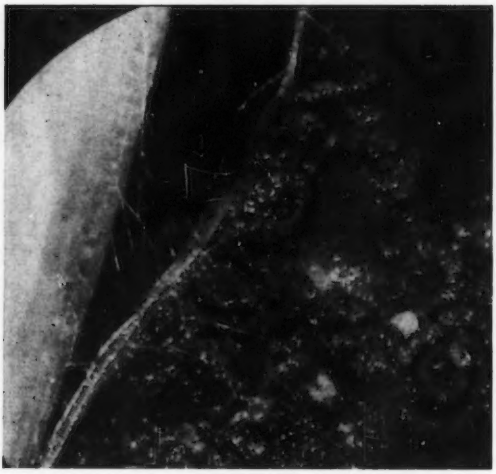

FIGURE 2. Hyphae of *H. sativum* growing from a wheat leaf lesion into and over adjacent soil and debris. (X200).

Spread of H. sativum from a Lesion or an Inoculation Focus into Adjacent Soil

Specimens of crowns from field collections of Thatcher and Saunders wheat were selected for these observations. They had been in the herbarium 18 months. Crowns showing slight and severe lesions were used. From 5 to 13 roots were selected from each crown. Care was taken not to disturb the clump of soil and root hairs enveloping the root for most of its length. In a day or so mycelial strands appeared, ramifying the soil, and after 7 days records were made on the number of roots showing sporulating *H. sativum.* The results from these collections and from greenhouse specimens, handled in a comparable manner, were as follows:

Collection	Variety	Number of		
		Crowns	Crown roots	Roots showing *H. sativum*
Hoey, 1958	Thatcher	10	78	31
Albertville, 1958	Thatcher	7	42	8
Shipman, 1959	Saunders	5	32	8
Greenhouse crop	Marquis	10	68	32

In general, crowns with severe lesions gave heavy sporulations of *H. sativum.* However, occasionally crowns without distinct lesions also produced sporulations.

Another technique was employed to determine the spread of *H. sativum* from roots. In earlier work it was found that dry wheat leaves were readily colonized by this fungus.* Therefore, 2 crown roots with adhering soil were selected at random from 10 plants of an Albertville collection and 10 from a Smeaton collection. These were laid on pieces of dry wheat leaf on moist filter paper in a Petri dish and incubated for 4 days at room temperature. Fungus growth was evident early and spread to the adjacent leaf where sporulating *H. sativum* was readily determined. There was, of course, other fungal growth as well. The results were as follows:

Collection	Number of		
	Crowns	Crown roots	Roots showing *H. sativum*
Albertville, 1958	10	20	17
Smeaton, 1959	10	20	9

*Annual repor., 1954. *Unpublished.*

This method seems quite feasible as a selective isolation technique for *H. sativum.* It shows that the mass of soil around the root and its microbial content do not prevent the fungus, when well established as in a lesion, from spreading and colonizing adjacent leaf tissue.

Further tests gave additional information on the spread of *H. sativum.* For example, crown pieces, including 3 or 4 inches of the culm and stubs of crown and seminal roots, were taken from Marquis plants grown in the greenhouse. These plants had some actively growing tillers but only the main culm was used. All crowns selected showed good lesions as a result of earlier inoculations with *H. sativum.* The specimens were laid on a layer of the soil from the crocks in which they grew and incubated at room temperature in Petri dishes. The specimens were observed for a week or so. An abundant fungal growth, much of which was *H. sativum,* spread rapidly from culm, leaf base tissues and crown root stubs to adjacent tissue. The ramifying hyphae spread over and through nearby soil clumps causing a distinct webbing. Sporulation of *H. sativum* was observed on the tissues and in the webs. Moreover, some of the hyphae were picked off and plated with positive results. A similar test with Marquis seedlings bearing lesions was conducted with the same results (Figure 2). In both trials other fungi appeared but of these only *Alternaria* was identified. Several tests of the above type were conducted with confirmatory results.

Pieces of inoculated wheat seedling leaves were also observed. They were laid on moist unsterilized soil in a Petri dish and covered almost entirely by a thin layer of soil. The inoculation was heavy. The dishes were kept at room temperature. A rapid colonization soon started and in a short time hyphae were ramifying the covering soil layer. In 7 days a good growth had been established and *H. sativum* was isolated by plating bits of the ramifying hyphae.

In a similar test the leaves were not covered with soil so that rapid colonization and spread could be observed. In 2 or 3 days the fungus had spread to nearby uninoculated pieces, a distance of approximately 5 millimetres, and in 7 days had spread as much as 20 or 25 millimetres from the source. In these tests on soil, as in earlier tests on moist filter paper, the colonized portion of the leaf retained its deep green colour while adjacent parts showed chlorosis.

The above experiments indicate that *H. sativum* under suitable conditions develops rapidly on wheat plant tissues and spreads readily from such origins over and through unsterile soil. These observations are of particular interest in view of the inhibitory influence of the soil flora on the germination of spores of *H. sativum* (2).

DISCUSSION

In the first contribution covering results of inoculation tests on detached wheat leaves (3), two reactions to infections by *H. sativum* were emphasized, the movement of nutrients, especially nitrogen, to the infection site and the apparent change in permeability of cell membranes. The latter reaction would facilitate the leaching of substances from the affected cells.

Metabolic changes of this nature are, of course, of prime pathological significance. Both of these phenomena have been studied by other workers using various fungal pathogens, as previously cited (3). The present work gives support to the concept of nitrogen migration towards the infection zone. When a severe and extensive lesion developed over the coleoptile area of a seedling, involving the base of the first leaf, chlorosis of the leaf blade soon appeared and the leaf died. In experiments with seedlings maintained by water, infections developing over a period of about 7 days caused a loss of one leaf in most cases. This loss would represent roughly one-third of the photosynthetic tissues in seedlings with three leaves. A similar loss was shown for seedlings of several varieties in relation to severe lesions. The application of a weak nitrate solution greatly reduced chlorosis and leaf loss, although severe lesions did develop. It seems, therefore, that injury resulting from infections under these conditions is in a large measure prevented when there is an ample supply of nitrogen.

Occasionally a lesion has only a limited development indicating some form of host resistance, perhaps the exhaustion of a suitable carbon (carbohydrate) supply in the vicinity of the invasion. In such circumstances, *H. sativum* could well adjust its development towards a saprophytic activity and colonize the immediate external substrate in search of nutrients. It was found by experiments that an enriched external substrate did promote the growth of *H. sativum*, thus increasing the colonization in the lesion area. Tests have shown that *H. sativum* spreads readily from lesions and other sources into the adjacent area; hence such growth may represent an extension of the colonization forming a united colony of lesion thallus plus external thallus. The drainage of nitrogen from the host in the development of such an establishment could be many times that used within a limited lesion. The data show a greater injury in the form of chlorosis where nitrogen was deficient, especially when the external substrate was enriched. The slight reduction in dry weight may not be significant but it was an indicative trend in all tests. One can speculate that, as the nutrients varied under field conditions through the season, so would the activity of the pathogen.

Infections at the base of a wheat plant are exposed to a fluctuating environment, such as changes in temperature and moisture of both soil and air, accumulations of dead leaf tissues and so forth. The spread of *H. sativum* from an established source was not inhibited by adjacent soil, such as soil masses adhering to roots, etc. Therefore, lesions formed at the crown are favourably located for expansion, at an opportune moment, of both the parasitic and saprophytic phases of *H. sativum*. It may be that we have not given sufficient attention to the influence of an external thallus in infections of this type.

Histological observations* have disclosed the main seat of the invasion of a rootrot fungus to be the cortex of roots and subcrown internode, and the tissues of leaf bases. From these loci, contact with the exterior can be simple and extensive. Besides the influence of nitrogen drainage, there

*Annual Reports, 1951 and 1955. *Unpublished.*

may be considerable leaching of nitrogen and other substances from the necrotic tissues and these would affect all organisms in the vicinity of the crown. Thus, complications may arise under field conditions. Nevertheless, it is thought that a well-established colonization by *H. sativum* is a prime factor in the resultant pathology of the crown zone.

ACKNOWLEDGEMENTS

The author wishes to thank Virginia Fisher, Technician, who assisted with most experiments, and R. J. Ledingham and F. L. Dolezsar for help with the illustrations.

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A GRASS-ALFALFA MIXTURE COMPARED WITH CEREAL GRAINS FOR FODDER PRODUCTION UNDER SEMI-ARID CONDITIONS

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[Received for publication February 27, 1961]

ABSTRACT

From 1952 to 1960 at Swift Current, Saskatchewan, the performance of a grass-alfalfa mixture was compared to that of oats and of wheat on dry-land, principally from the standpoint of hay production. Average annual hay yields over the 9-year period were similar, but within single years large differences in yield occurred between crops. In seasons with favourable precipitation the perennial forage mixture gave higher hay yields, while the cereals gave the highest yields during very dry seasons.

Small yields from the perennial crop were associated with low rainfall in May. It was concluded that perennial forage crops should be the main source of winter feed in a semi-arid climate, if used in conjunction with a cereal crop for insurance.

INTRODUCTION

The production of forage for winter livestock feed is of major importance in the semi-arid region of the Canadian prairies. In this area climatic conditions are considered to be more favourable for growing cereals than for growing perennial grasses and legumes. As a result, cereals have been used extensively for the production of hay (7). This situation has developed partly because very little (1, 7) research information comparing the performance of annual and perennial crops for winter feed reserves has been reported.

Clarke (3) emphasized the problem of winter feed reserves in the area in 1935. Despite these and other exhortations, farmers as a whole have not adjusted their cropping practices to compensate for the increased demand for fodder, although Hopkins (5) had anticipated such would be the case when he also discussed potential change in primary production in 1935. More recently Thomson *et al.* (7) stressed the same importance. Since the 1930's the cattle population in the Prairie Provinces has increased from 3 to 5½ million head, with most of the increase occurring since 1950 (4). During this last decade of rapid increase in cattle population the acres in cultivated forage crops per animal has increased only slightly from ⅗ acre per animal to ⅔ acre per animal (4).

The need for information to compare the yields and performance of annuals and perennials as fodder was realized and subsequently tests have been established to determine relationships. This paper presents the data collected from a test conducted at Swift Current, Saskatchewan, during the 1952-1960 period.

MATERIALS AND METHODS

The test was conducted on a long-time (55 years) cultivated field of Wood Mountain loam in the Brown Soil Zone at Swift Current. This land had previously been used for growing cereal grains, primarily in a 2-year wheat-summerfallow rotation. Four treatments with four replicates made up the test in a latin square design. The treatments used in the test were: 1) brome-crested wheatgrass-alfalfa mixture, one seeding at 4, 3, and 2

TABLE 1. — DRY MATTER HAY YIELDS FOR PERENNIAL AND ANNUAL CEREAL CROPS AT SWIFT CURRENT, SASKATCHEWAN

Crop treatment	Pounds per cultivated acre									
	1952	1953	1954	1955	1956	1957	1958	1959	1960	9-year average
Perennial grass-alfalfa mixture	3340	4200	2760	3160	1400	500	160	820	800	1900
Oats — continuous cropping	2760	2460	1960	2000	1880	780	720	1520	1340	1720
Oats — summerfallow 2-year rotation	2440	2200	2880	2260	1900	1240	760	2140	1560	1940
Wheat — summerfallow 2-year rotation	2140	2180	2200	1620	1720	1160	960	1920	1600	1720
Mean	2680	2760	2440	2260	1720	920	640	1600	1320	
L.S.D. (P = .05)	N.S.	900	N.S.	360	N.S.	380	280	600	420	
S.E.M. %	11.9	10.1	15.4	5.0	7.8	13.0	8.5	11.7	10.0	

TABLE 2. — PRECIPITATION DISTRIBUTION DURING 1952 TO 1960 AT SWIFT CURRENT, SASKATCHEWAN

Year	Inches of precipitation		
	May	May & June & July	Annual
1952	1.87	7.95	16.05
1953	2.77	7.46	15.63
1954	3.31	10.11	19.71
1955	2.21	8.27	17.31
1956	1.73	7.14	13.15
. . . . .	. . . . .	. . . . .	. . . . . [1]
1957	0.15	5.00	11.80
1958	0.47	3.51	11.50
1959	0.65	6.68	13.99
1960	0.98	5.37	12.44

[1]Dotted line divides years of good May precipitation from those of poor May precipitation.

pounds per acre of the three species in rows 12 inches apart; 2) continuous oats seeded each year on same plots at a 2-bushels-per-acre seeding rate; 3) 2-year oats-summerfallow rotation at 2-bushels-per-acre seeding rate; and 4) 2-year wheat-summerfallow rotation seeded at 1¼ bushels per acre. Rows in all cereal plots were spaced 6 inches apart. One-half of the plot area in treatments 3 and 4 was seeded each year, while the other half was summerfallowed in preparation for the next year's seed bed. Large plots, 40 x 68 feet, were used to facilitate the use of field machinery in the annual summerfallowing practice and annual seeding treatments.

Varieties of wheat and oats used each season was one officially recommended for the area by the Saskatchewan Advisory Council on Grain Crops. Because of changes in the official recommendation different varieties were used during the course of the experiment. The wheats used were Rescue, Chinook, and Selkirk, while the oat varieties were Ajax, Fortune, Garry, and Rodney.

The experiment was started in the spring of 1951. The grass-alfalfa mixture and the continuous oat plots were seeded in that year while the other two treatments were summerfallowed. This allowed collection of data to commence in 1952.

Forage yields were determined each year by cutting a sample 3 feet x 31 feet out of the centre of each plot. The grass-alfalfa mixture was harvested near the first of July when alfalfa was in the mid-bloom stage. The cereal grains were cut when the kernels were in the soft dough stage. Usually this entailed cutting the oats during the first week of August and the wheat a few days later. A portion of each cereal plot was left to mature, after which 3-square yard samples within each plot were harvested for grain yield determinations.

To obtain equitable comparisons between treatments, the yields per acre for the 2-year rotations were calculated on the basis of total area of

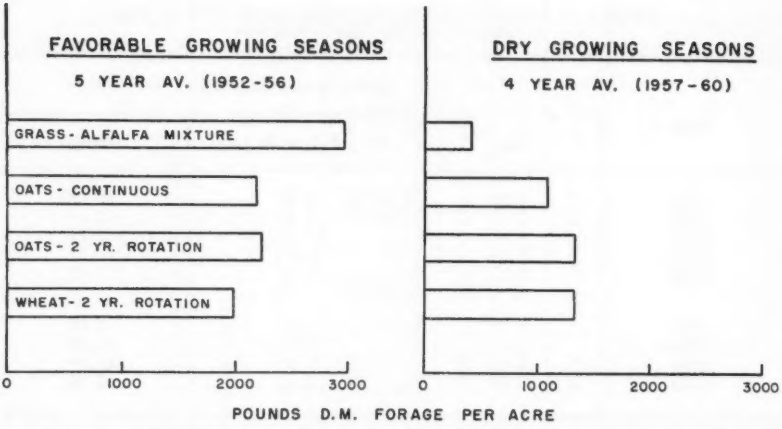

FIGURE 1. Hay yield comparisons during favourable seasons and during drier seasons showing advantage of perennial mixture in good seasons and of cereals in dry seasons.

the planted crop and the summerfallow in the plot. That is, the initial productivity of the cereals grown on summerfallowed soil (one-half of the plot) was halved before analysis and interpretations. April-October precipitation was measured with a rain gauge located at the test site.

RESULTS AND DISCUSSION

The mean dry matter hay yield for each crop in each year is shown in Table 1. The average yield over the 9-year period differed very little between the crops, but there were obvious interactions between the crops and years. During the favourable years, 1952 to 1956, inclusive, the yield from the perennial mixture was comparable to or greater than those obtained from either of the cereal crops in either cropping procedure in all years. On the other hand, during the dry years 1957 to 1960, inclusive, the yields from the perennial grass-alfalfa mixture were less than those obtained from the cereal crops grown in either cropping procedure for all years.

To illustrate the yield performance of the crops during the two aforementioned periods, the mean values as determined for those two periods are shown in Figure 1. The classifications "Favourable Growing Seasons" and "Dry Growing Seasons" were based largely on distribution of rainfall, but also to some extent on annual precipitation. The rainfall recorded at the test site during the month of May, and during the 3-month period (May-June-July), along with the total annual precipitation measured at Swift Current for each year, are shown in Table 2. A comparison of the May rainfall in particular with the yield values in Table 1 will indicate the association between low precipitation and low yield, especially for the perennial crop. This association between low May rainfall and low yields of perennial forages has been recognized for many years (2, 6) and suggests a reason for former reliance on cereal crops for hay production.

TABLE 3. — GRAIN YIELDS FOR EACH CEREAL CROP TREATMENT AT SWIFT CURRENT, SASKATCHEWAN

	Grain yields — pounds per cultivated acre									
	1952	1953	1954	1955	1956	1957	1958	1959	1960	9-year average
Oats — continuous cropping	2790	1370	1180	1530	1430	1210	600	1080	480	1300 (38 bu.)
Oats — summerfallow — oats rotation	2180	1170	1550	1340	990	1000	470	750	570	1110 (32 bu.)
Wheat — summerfallow — wheat rotation	1110	980	750	920	730	830	440	600	670	780 (13 bu.)
Mean	2030	1170	1160	1280	1050	1010	500	810	570	
L.S.D. (P = .05)	560	N.S.	200	230	190	290	N.S.	N.S.	N.S.	
S.E.M. %	7.9	2.7	4.8	5.2	5.2	8.1	11.6	20.0	10.8	

Table 3 shows the mean grain yields obtained from each cereal treatment throughout the 9 years. It should be noted that, when comparisons were made on a per cultivated-acre basis, continuous cropping resulted in oat yields which were as great as or greater than those from rotational cropping in all years except 1954.

CONCLUSION

It is evident from the foregoing that conclusive comparisons between perennial forage mixtures and annual cereal grains can only be ascertained within a specified period of time. Climatic factors, in particular precipitation and its seasonal distribution, have a marked effect on yielding ability of different crops. Low rainfall in May puts the perennial forage crop at a disadvantage. However, over the 9 years of the test, 4 of which were of low rainfall in May, the perennial mixture yielded as much hay as the cereals. Thus the two crop types should be regarded as complementary rather than alternate sources of forage. There is good reason to believe that an adequate acreage of a perennial forage mixture should provide on the average less expensive and better quality livestock feed, but that some cereal grains, preferably oats, should be planted yearly as an insurance against a poor season for the perennial crop.

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SOME EFFECTS OF GAMMA RADIATION ON STORED ONIONS[1]

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[Received for publication May 3, 1961]

ABSTRACT

Gamma radiation doses of 3200, 4700, 6300, 7900 and 9500 rad completely inhibited sprouting of Brigham Yellow Globe onions in a 300-day test period during which 75 per cent of the non-irradiated controls sprouted. In a second test of equal duration, using controlled temperature storage and shed storage, less than 3 per cent of Autumn Spice onions exposed to either 4000 or 8000 rad sprouted, whereas more than 79 per cent of the non-irradiated bulbs sprouted. Irradiation reduced sprouting of Yellow Sweet Spanish onions in both tests.

Some evidence indicated that the smaller bulbs in both the irradiated and non-irradiated lots were less susceptible to storage rot. In Brigham Yellow Globe, the incidence of internal discoloration of the growing point was greatest with the highest radiation dose. Irradiated onions were milder in flavour.

INTRODUCTION

For many years onions have been stored successfully for limited periods by maintaining a low temperature and dry atmosphere and providing adequate ventilation in the storage room. Recent researchers have used pre-harvest chemical sprays to prolong the storage life of onions (5, 7, 9). Maleic hydrazide is the most effective of these, but concentration and timing of application were shown to be critical because of possible injury to the plants or bulbs (5, 7, 9). Probably for this reason, chemical treatment for sprout control has not been widely practised in Canada.

More recently, pre-storage irradiation of cured onions has proved effective in controlling sprouting over a period of several months (1, 2, 3, 4, 6, 10). Reports 3 and 4 appeared after the observations reported herein were made.

MATERIALS AND METHODS

1957-58

A preliminary study of the effect of pre-storage gamma radiation was begun in November, 1957. Cured bulbs of Yellow Sweet Spanish and Brigham Yellow Globe were used. The former, a late-maturing variety, had been mechanically topped. Before packaging, the onions were separated into large and small size categories. Large bulbs of Yellow Sweet Spanish measured more than 3.4 inches in equatorial diameter. Large bulbs of Brigham Yellow Globe measured more than 2.8 inches. Populations of each size and for each irradiation exposure averaged 30 bulbs. These were not replicated. Each lot was packaged in a half-bushel, rectangular, corrugated cardboard carton lined with a polyethylene bag which was closed with filament tape.

The onions were shipped to the Atomic Energy of Canada, Limited, Chalk River, Ontario, where they were exposed at the spent fuel rod gamma irradiation facility. Five radiation doses ranging from 3200 to 9500 rad, at an average dose rate of 32,000 rad per hour, were used.

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After treatment, the irradiated and non-irradiated bulbs were transferred at Ottawa to shallow, slatted crates and placed in controlled temperature storage at 55°F. and 75 per cent relative humidity. The humidity was higher than that recommended for onion storage. Observations on storage behaviour were made at 4- to 6-week intervals.

1958-59

Yellow Sweet Spanish was retained for continued study but Brigham Yellow Globe was replaced by Autumn Spice, the chief hard onion in current Canadian production. Unreplicated lots of 150 cured bulbs of relatively uniform size were exposed to either 4000 or 8000 rad of gamma radiation at the rate of 288,000 rad per hour. This was done in a Gammacell 220, a Cobalt 60 irradiation facility at the Commercial Products Division, Atomic Energy of Canada, Limited, Ottawa, Ontario. Comparable check lots were left untreated. The onions were transported and stored in slatted half-bushel crates. For 200 days the temperature was held at 48°F. and the relative humidity at 70-75 per cent. The temperature was then raised to 68°F.

Irradiated and non-irradiated lots of Autumn Spice were also placed in shed storage where comparatively early sprouting would be expected. In this cork-insulated room, fitted with hand-manipulated ventilators, the temperature throughout the winter months fluctuated from 34° to 64°F. During the spring and summer months it was slightly lower than that outdoors and ranged from 50° to 80°F. The atmosphere in the shed storage room was very dry, particularly during the winter and spring periods.

At each inspection in both years, bulbs were classified into one of the three categories: sprouted, rotted, and sound. Those showing vigorous sprout development were classified as *sprouted*; unsprouted bulbs showing evidence of decay were classified as *rotted;* bulbs showing no sprouting or rotting were rated as *sound.* Rotted bulbs were discarded at each inspection to avoid contaminating sound bulbs. Sprouted bulbs in irradiated lots were retained for further observation. Sprouted non-irradiated bulbs were discarded without being examined for internal rotting if the sprouts appeared normal and vigorous. Examination in longitudinal section of all apparently sound bulbs was made at the end of the 300-day storage.

RESULTS AND DISCUSSION

Inhibition of Sprouting

Data for lots subjected to different irradiation doses in both years were combined at the termination of storage because there was no evidence of differences between them in the amount of sprouting (Table 1). Irradiation in 1957-58 completely inhibited sprout development in Brigham Yellow Globe onion for the entire 300-day storage period. Seventy-five per cent of the non-irradiated bulbs sprouted normally during that time. After 300 days of storage in 1958-59, sprouting was negligible in irradiated Autumn Spice onions in both storage rooms. Prior to raising the thermostat-controlled storage temperature during 1958-59, non-irradiated bulbs of Autumn

FIGURE 1. Effect of gamma radiation on sprouting of Autumn Spice onion following 300 days in shed storage.

FIGURE 2. *Left to right*: Normal sprouting in a non-irradiated onion; sprout "die-back" in an irradiated bulb; and an unsprouted irradiated bulb.

TABLE 1. — CUMULATIVE PERCENTAGES OF SPROUTED, ROTTED, AND SOUND BULBS IN IRRADIATED AND NON-IRRADIATED ONIONS AFTER THE INDICATED STORAGE PERIODS

Year	Variety	Treatment	Storage period								
			100 days			200 days			300 days		
			Sprouted[2]	Rotted	Sound	Sprouted	Rotted	Sound	Sprouted	Rotted	Sound
1957-58	Yell. Sw. Spanish	Irradiated	6.9	30.8	62.3	10.5	65.4	24.1	11.1	81.3	7.6
		Non-irradiated	15.3	37.5	47.2	51.2	47.0	1.8	53.0	47.0	0.0
	Brig. Yell. Globe	Irradiated	0.0	4.3	95.7	0.0	10.7	89.3	0.0	24.0	76.0
		Non-irradiated	0.0	3.3	96.7	11.7	6.0	82.3	75.0	10.0	15.0
1958-59	Yell. Sw. Spanish	Irradiated	0.0	38.3	61.7	0.0	60.3	39.7	1.3	87.0	11.7
		Non-irradiated	0.0	60.6	39.4	3.3	76.0	20.7	20.0	79.0	1.0
	Autumn Spice	Irradiated	0.0	1.4	98.6	0.7	4.7	94.6	1.3	16.0	82.7
		Non-irradiated	0.0	5.3	94.7	2.7	8.0	89.3	79.0	9.3	11.7
		Irradiated[1]	1.6	1.0	97.4	3.0	1.4	95.6	3.0	15.0	82.0
		Non-irradiated[1]	3.3	1.3	95.4	53.3	3.3	43.4	94.0	4.0	2.0

[1]These two lots were held in shed storage.
[2]All sprouting which occurred in irradiated bulbs was weak in vigour and died back.

TABLE 2. — EFFECT OF BULB SIZE ON KEEPING QUALITY IN IRRADIATED AND NON-IRRADIATED BULBS OF TWO ONION VARIETIES UNDER CONTROLLED TEMPERATURE AND HUMIDITY DURING THE 1957-58 STORAGE SEASON

Variety	Bulb size	Treatment	Storage period								
			100 days			200 days			300 days		
			Sprouted %	Rotted %	Sound %	Sprouted %	Rotted %	Sound %	Sprouted %	Rotted %	Sound %
Yell. Sw. Spanish	Small	Irradiated	6.6	31.5	61.9	11.1	60.7	28.2	11.1	79.2	9.7
	Large		7.0	30.0	63.0	9.8	70.2	20.0	9.8	83.4	6.8
	Small	Non-irradiated	18.5	29.6	51.9	63.0	33.3	3.7	66.7	33.3	0.0
	Large		12.1	45.4	42.5	39.4	60.6	0.0	39.4	60.6	0.0
Brig. Yell. Globe	Small	Irradiated	0.0	2.3	97.7	0.0	6.5	93.5	0.0	21.6	78.4
	Large		0.0	6.9	93.1	0.0	16.1	83.9	0.0	27.2	72.8
	Small	Non-irradiated	0.0	0.0	100.0	8.6	0.0	91.4	74.3	0.0	25.7
	Large		0.0	8.0	92.0	16.0	12.0	72.0	76.0	20.0	4.0

Spice kept well but, following 100 days at 68°F., 79 per cent sprouted, 9.3 per cent rotted, and only 11.7 per cent remained sound. Figure 1 shows the effectiveness of both radiation doses in controlling sprouting of Autumn Spice under shed storage conditions.

In both years, irradiation reduced sprouting of Yellow Sweet Spanish (Table 1). However, severe rotting in this variety started early in both storage periods. After 200 days, more than one-half of the bulbs had been discarded. Probably a large proportion of the non-irradiated bulbs which rotted would otherwise have sprouted.

Only weak sprouts developed on irradiated bulbs of Yellow Sweet Spanish and Autumn Spice. These sprouts withered and died back after attaining a length of only 1 to 3 inches (Figure 2), and the bulbs rotted before the end of the storage period. Sprout "die-back" in irradiated produce has also been reported by others (1, 8).

Sound Bulbs

A very high percentage of irradiated Brigham Yellow Globe and Autumn Spice onions were sound after 300 days of storage (Table 1). After 100 days' storage in both tests, more than 60 per cent of irradiated Yellow Sweet Spanish bulbs were in apparent marketable condition. Less than 45 per cent of the non-irradiated were sound. Although Yellow Sweet Spanish is recognized as a "soft", relatively poor keeping onion, it would appear that pre-storage irradiation may be of some use in extending its otherwise short storage life.

It is particularly noteworthy that irradiated Autumn Spice stored as satisfactorily and for as long a period in shed storage as in controlled temperature storage. In shed storage more than 50 per cent of the non-irradiated bulbs had sprouted after 200 days, and 94 per cent after 300 days. It would appear, therefore, that, in addition to lengthening the storage life of onions, irradiation might reduce storage costs by eliminating the need for close temperature control.

At the end of the storage period, sound bulbs in both the irradiated and non-irradiated lots were firm, with skins relatively intact and bright in colour. After a close examination, they were all classified as marketable onions.

Storage Rot

A watery exudation from the neck of the onion typified most of the rotting in the irradiated and non-irradiated lots of all three varieties in both seasons. The cause of rotting was not determined. It is possible that irradiation may have induced rotting as a result of damage to the meristem.

After 100 days, rotting was most frequent in the non-irradiated onions (Table 1), but after 300 days a higher percentage of the irradiated bulbs had rotted. However, the data before combining exposure doses did not indicate that rotting was associated with either the high or low levels of radiation. Possibly a proportion of the sprouted non-irradiated bulbs that were discarded without being examined internally were rotting. This would account for the apparent greater rotting in the unsprouted irradiated bulbs.

TABLE 3. — INCIDENCE OF INTERNAL DISCOLORATION AND DECAY IN LONGITUDINALLY SECTIONED EXTERNALLY SOUND[1] UNSPROUTED, IRRADIATED AND NON-IRRADIATED BULBS OF THREE ONION VARIETIES FOLLOWING 300 DAYS OF STORAGE

Year	Variety	Irradiation dose	Bulbs examined No.	Internally discolored		Internally decayed		Clear	
				No.	%	No.	%	No.	%
1957-58	Brig. Yell. Globe	Control	10	0	0.0	0	0.0	10	100.0
		3200 rad	48	6	12.5	0	0.0	42	87.5
		4700 rad	46	8	17.4	0	0.0	38	82.6
		6300 rad	50	14	28.0	1	2.0	35	70.0
		7900 rad	41	11	26.8	1	2.4	29	70.8
		9500 rad	43	13	30.2	1	2.3	29	67.5
1958-59	Yell. Sw. Spanish	Control	1	0	0.0	0	0.0	1	100.0
		4000 rad	21	9	42.8	3	14.4	9	42.8
		8000 rad	16	6	37.5	0	0.0	10	62.5
	Autumn Spice	Control	19	4	21.0	3	15.8	12	63.2
		4000 rad	128	16	12.5	6	4.7	106	82.8
		8000 rad	120	17	14.2	9	7.5	94	78.3
		Control[2]	3	2	66.7	0	0.0	1	33.3
		4000 rad[2]	126	57	45.2	12	9.6	57	45.2
		8000 rad[2]	123	50	40.7	2	1.6	71	57.7

[1]Bulbs examined in longitudinal section were firm and in apparent marketable condition.
[2]These three lots were held in shed storage.

Table 1 shows that the varieties differ in susceptibility to storage rot. Because Yellow Sweet Spanish onions used in this experiment were mechanically topped, prior to curing, they may have been more susceptible to fungus invasion through the uncured neck. This and high humidity in the storage room probably were largely responsible for the unusually severe rotting in that variety. Much less rotting occurred in the other two varieties in the same storage. Rotting was least in Autumn Spice in shed storage where the humidity was low.

Effect of Size on Keeping Quality

Except for irradiated bulbs of Yellow Sweet Spanish rotting was greatest in the large bulbs in 1957-58, whether or not they were irradiated (Table 2). Both sizes of Yellow Sweet Spanish irradiated bulbs had a high percentage of storage rotting after 100 days. In both varieties, the non-irradiated lots had the greatest spread in per cent rotting between the large and small bulbs. It is noteworthy that none of the small non-irradiated bulbs of Brigham Yellow Globe rotted during the entire storage period. If small size was associated with earlier maturity in the test onions used, the smaller bulbs probably were more completely cured than the larger bulbs at the time of exposure and initial storage, and so should store better.

Table 2 also indicates varietal differences in the non-irradiated lots in relative susceptibility of the large and small bulbs to storage sprouting. Small non-irradiated bulbs of Yellow Sweet Spanish sprouted more freely than did the large bulbs, but this may have resulted from the lower incidence of rotting in the small bulbs. In contrast, the percentage of sprouting in the non-irradiated bulbs of Brigham Yellow Globe was still small after 200 days and was lowest in the smaller bulbs. After 300 days, it was high in both size categories.

Internal Discoloration

Discoloration of the interior of the bulb resulting from injury to or death of the growing point was observed in longitudinal sections of apparently sound marketable bulbs. To avoid portraying an exaggerated picture these results are given as actual counts as well as in per cent (Table 3).

"Internal discoloration" refers only to those bulbs in which the growing point had darkened and appeared dead. The internal decay referred to in Table 3 is presumably the early stage of storage rot and may or may not be secondary to the death of the growing point caused by irradiation or other means. The extent of this decay varied, but it was consistently located in the meristematic tissue.

After 300 days of storage in 1957-58, internal discoloration in Brigham Yellow Globe, with one exception, increased in percentage with radiation. There were too few sound unsprouted Yellow Sweet Spanish for comparison. In 1958-59, there was little difference between the effect of radiation doses of 4000 and 8000 rad on the amount of internal discoloration in Yellow Sweet Spanish, and in Autumn Spice in either storage room.

Internal discoloration in Autumn Spice was greater in shed-stored bulbs than in those stored at controlled temperatures.

Discoloration found in the control lots of Autumn Spice appeared identical with that found in irradiated bulbs of all three varieties, suggesting that irradiation merely intensifies a pre-existing condition in untreated bulbs. Dark centres in irradiated onions were reported by other workers (3, 4). Dallyn and Sawyer (4) suggested that this may not be objectionable to some users if the alternative is a sprouted onion.

Effect on Flavour

At the end of the 1958-59 storage period, random samples of unsprouted irradiated and non-irradiated bulbs of Autumn Spice were peeled and cooked. Results of a taste panel conducted by the Food Processing Section of the Plant Research Institute, Central Experimental Farm, Ottawa, revealed that the irradiated onions had a noticeably milder flavour than the non-irradiated controls. The panel did not detect flavour differences between the two radiation doses. Loss of characteristic flavour – particularly sharp or astringent qualities – in irradiated produce was reported by Hannan (6). In the present experiment, no off-flavour was detected in either the irradiated or non-irradiated lots. A similar observation was made by others (4).

CONCLUSIONS

These experiments showed that a radiation dose as low as 3200 rad gives satisfactory sprout control in hard onions for up to 300 days. A slightly higher dose was effective for the same duration when the only temperature control was to prevent freezing or temperatures higher than 70°F. in winter. Under such conditions, non-irradiated onions sprouted readily. Thus, in addition to lengthening storage life of onions, irradiation might reduce storage costs by eliminating the need for close temperature control.

The sprout-inhibiting effectiveness of a low radiation dose is also significant because of evidence that higher doses cause excessive internal discoloration. The relation of internal discoloration to irradiation is not clear, however, because discoloration occurred also in some control lots. This point should be investigated further.

Varieties differed in susceptibility to storage sprouting and rotting. Although late maturity and high storage humidity may have increased rotting of the Yellow Sweet Spanish onion, this variety is known to have a relatively poor storage quality. It appears, however, that irradiation is of some use in extending its storage life. More varieties should be compared.

Although irradiation tends to mellow the flavour of the onion, it is unlikely that it has an adverse effect on culinary quality. This agrees with the findings of others.

The results obtained during this 2-year period justify further study of irradiation-inhibition of sprouting in stored onions. Points needing investigation, in addition to those already suggested, are the effect of lower radiation doses, a comparison of different storage conditions, and a study of the

economic feasibility of commercial irradiation of onions based on pilot plant operation.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the helpful suggestions and constructive criticisms given by A. W. S. Hunter and D. R. Sampson, Genetics and Plant Breeding Research Institute, in the preparation of this manuscript.

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FATTY ACID COMPOSITION OF FLAX VARIETIES[1]

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[Received for publication March 16, 1961]

ABSTRACT

Iodine number and fatty acid composition of linseed oil from flax varieties grown in Western Canada are reported. Differences in iodine number and linolenic acid due to environment were greater than differences between varieties grown in the same location, but varieties tended to maintain their respective rank in regard to fatty acid composition within broad limits regardless of environment.

INTRODUCTION

Linseed oil is the drying oil most commonly used in industry for the manufacture of paint and similar coatings. Its drying properties, like those of other drying oils, depend on the polyunsaturated linolenic and linoleic acids it contains. This paper reports the range of fatty acid composition for oil from a number of flax varieties grown in Canada and shows the effect of variety and environment on fatty acid composition for five varieties grown at two widely separated localities in Western Canada.

MATERIALS AND METHODS

Oil from composite seed samples of varieties and hybrid selections of linseed flax grown at eight stations (Morden, Portage la Prairie, Brandon and Gilbert Plains in Manitoba, and Indian Head, Melfort, Scott and Swift Current in Saskatchewan) in 1955, 1956 and 1957 was analysed for iodine number and fatty acid composition. A composite sample of seed of each variety was made up from all stations and a composite for each station of all varieties. Of the four check varieties, Raja and Redwing are earlier in maturity than Bison and Redwood, whereas Raja and Bison are known to be lower in iodine number than Redwing and Redwood. In addition, five varieties (Raja, Redwing, Redwood, Rocket and Marine) grown at Morden (49° 11′N) and Fort Vermilion (58° 25′N) were analysed separately to determine the effect of climate on oil content, and iodine number (total unsaturation) and fatty acid composition of the oil. For all samples, oil, separated by pressing, was analysed for iodine number by the method of Hiscox (3) and for fatty acid composition by the A.O.C.S. tentative method for polyunsaturated acids (Cd. 7-48, rev. 1953) as modified by Vandenheuvel and Richardson (6).

RESULTS AND DISCUSSION

Iodine number and unsaturated fatty acid composition of oil for the check varieties and a group of hybrids grown at eight stations are given in Table 1. The hybrids were chosen to represent some of the extreme values found. Iodine number ranged from 182 to 188. Linolenic acid varied from 46.2 to 54.6 per cent and was closely correlated (+ .86) with

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TABLE 1. — IODINE NUMBER AND UNSATURATED FATTY ACID COMPOSITION OF OIL OF COMPOSITE SAMPLES OF FLAX VARIETIES AND HYBRIDS GROWN AT EIGHT STATIONS IN WESTERN CANADA. AVERAGE FOR 1955-57.

Variety	Iodine number	Linolenic acid	Linoleic acid	Oleic acid
	Wijs	%	%	%
Bison	185	47.6	14.1	31.8
Raja	185	48.2	14.8	27.3
Redwing	194	52.9	14.4	25.4
Redwood	193	53.1	13.5	24.6
R.L. 51	198	51.5	14.0	35.4
M311-8	196	54.6	11.3	29.8
M468-16	194	52.9	13.9	29.0
Ott. 4693A	192	52.3	14.6	24.4
EG48-55	191	52.5	11.9	28.8
R.L.219	190	51.5	14.2	26.1
R.L.119	188	51.4	12.0	28.5
M307-151	185	50.8	10.9	28.8
I.H.4224	184	46.2	16.5	31.2
M326-153	182	46.9	14.7	30.2
Av. checks	189.4	50.5	14.2	27.3
Av. hybrids	191.3	51.2	13.7	29.2
S.E.	2.73	1.23	0.55	2.92

iodine number. This close relationship of iodine number and linolenic acid has already been observed (1, 4, 5). Oleic acid varied from 24.4 to 31.8 per cent and followed the inverse relationship with linolenic acid pointed out by Zimmerman and Klosterman (7). However, Zimmerman and Klosterman found no correlation between linoleic acid content and the iodine number, oleic, linolenic or saturated acids. In this study linoleic acid varied from 10.9 to 16.5 per cent and showed a significant negative correlation (— .37) with linolenic acid.

A study of the composite from each station had shown that oil of samples from Morden had a lower iodine value than those from the more northerly stations. Separate analyses of five standard varieties grown at Morden and Fort Vermilion provided an opportunity to study the effect of differences in environment on fatty acid composition in more detail.

Iodine number and linolenic acid (Table 3) showed highly significant differences between varieties and between stations. Iodine number and linolenic acid content differed an average of 24 points and 12 per cent respectively between stations in 1957 (Table 2). The values were higher at Fort Vermilion and the difference between stations was greater than the differences between varieties.

The variation between varieties has been shown to be due to Raja which was significantly lower in iodine number and linolenic acid content and higher in linoleic acid. The percentage difference due to location was also greater for Raja than for the other varieties. However, varieties tended to maintain the same relative order of fatty acid composition of oil each

TABLE 2. — IODINE NUMBER, LINOLENIC AND LINOLEIC ACID VALUE OF LINSEED OIL FROM FLAX VARIETIES GROWN AT MORDEN, MAN., AND FORT VERMILION, ALTA. 1955-57.

Variety	Morden, Man.				Fort Vermilion, Alta.			
	Oil content	Iodine No.	Linolenic acid	Linoleic acid	Oil content	Iodine No.	Linolenic acid	Linoleic acid
1955	%	Wijs	%	%	%	Wijs	%	%
Raja	37.9	172	42.9	16.5	39.3	198	55.0	15.2
Redwing	36.9	186	49.2	14.1	38.7	205	60.4	15.1
Redwood	39.8	184	49.5	13.4	42.6	204	58.5	14.0
Rocket	39.0	181	47.1	13.0	41.5	202	59.0	14.7
Marine	39.0	185	49.0	13.2	40.4	207	60.7	13.2
Av.	38.5	180	46.9	13.8	40.7	203	58.4	14.1
1956								
Raja	39.0	185	48.4	14.0	41.4	193	52.9	14.8
Redwing	37.7	194	52.1	14.2	—	198	55.9	14.5
Redwood	39.4	188	52.3	10.6	38.8	198	56.1	11.9
Rocket	39.5	190	51.6	11.6	41.0	198	55.1	13.0
Marine	39.1	194	5.33	12.3	40.8	202	58.0	12.9
Av.	39.3	189	51.3	12.5	40.4	197	55.3	13.6
1957								
Raja	—	171	43.0	13.9	—	200	55.8	14.2
Redwing	—	186	49.2	11.1	—	206	58.5	13.6
Redwood	—	182	48.3	12.9	—	205	62.2	10.5
Rocket	—	184	49.8	12.1	—	204	60.9	12.3
Marine	—	187	51.5	11.6	—	205	60.3	13.4
Av.	—	180	47.5	12.3	—	204	59.9	12.7

TABLE 3. — ANALYSES OF VARIANCE FOR MEAN SQUARES OF FIVE VARIETIES OF FLAX GROWN AT MORDEN, MAN., AND FORT VERMILION, ALTA. 1955-57.

Source of variation	D.F.	Iodine number		Linolenic acid		Linoleic acid	
		M.S.	F.	M.S.	F.	M.S.	F.
Stations	1	2188.80	1022.49**	579.04	432.12**	2.08	2.57
Years	2	4.94	2.98	1.69	1.26	7.05	8.70**
Varieties	4	102.64	47.29**	30.41	22.69**	6.39	7.89**
Raja vs. others	1	357.08	164.55**	113.69	84.84**	16.36	20.20**
Others	3	14.49	6.67*	2.65	1.98	3.06	3.78
Stations vs. years	2	158.80	73.18**	42.97	32.07**	0.24	0.30
Stations vs. varieties	4	11.47	5.29*	0.68	0.51	0.54	0.67
Years vs. varieties	8	1.93	0.89	1.24	0.93	0.52	0.64
Years vs. stations vs. varieties	8	2.17		1.34		0.81	

*Significant to 5% level
**Significant to 1% level

year at each station. The same was true each year for the composite samples reported in Table 1. The crop grown at Fort Vermilion had a higher oil content than that at Morden, the difference being as high as 2 per cent for some varieties.

Linoleic acid values varied over a shorter range and were significantly different between varieties but not between stations. In this study linoleic acid in contrast to linolenic acid value differed significantly between years.

It has been observed (Hilditch 2), that linseed when grown in a cool climate produces oils of a more unsaturated character than when grown in a warmer locality. Similarly Dillman and Hopper (1) found a higher oil content in a cooler climate. These relationships have been substantiated in this study. Mean temperatures were 3° to 13°F. cooler at Fort Vermilion for each month of the growing season than at Morden. This caused slower growth, and 20 to 30 days' later maturity at Fort Vermilion as compared to Morden.

These data demonstrate that linseed flax grown in northern areas has a higher oil and linolenic acid content than that grown farther south. They also show that varieties may differ in oil content and oil composition. However, since they tend to rank in the same order for these characteristics under different environments the plant breeder has an opportunity to produce a better variety regardless of his location.

ACKNOWLEDGEMENT

The authors wish to acknowledge the help of B. C. Stone, who carried out most of the chemical analyses.

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THE RESPONSE OF RAJA AND REDWING FLAX TO TEMPERATURE AND TO SUPPLEMENTARY INCANDESCENT LIGHT IN GROWTH CHAMBERS[1]

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[Received for publication April 12, 1961]

ABSTRACT

Raja and Redwing flax have a similar maturity rating in Southern Canada but differ considerably when grown near or north of the 55th parallel. To find an explanation for this difference, plants of both varieties were grown to maturity under two sets of light conditions (fluorescent lights alone and fluorescent plus incandescent lights) of identical light intensity at temperatures of 70° and 80°F. The varieties were found to differ significantly in time of blossoming, elongation and rate of growth in response to the treatments. The suggestion is made that the differences in response can be used by the plant breeder to create better adapted and more productive synthetic varieties.

INTRODUCTION

Raja and Redwing are early maturing varieties of flax, *Linum usitatissimum* L., with similar maturity ratings in Southern Canada and the North-Central United States. However, Raja matures on the average 7 days later than Redwing in Canadian agricultural areas near and north of the 55th parallel of latitude (3).

Many climatological phenomena influence the development of annual plants. Carder (2) compared the effect of some of these on wheat, barley, oats, peas and millet, at Madison, Wisconsin (latitude 43° N.), and at Beaverlodge, Alberta (55° N.), and found that temperature and length of day (photoperiod) were mainly responsible for the differential growth behaviour of the crop varieties at these locations. He also found that Beaverlodge received about the same amount of insolation as Madison, and that the mean monthly day-to-day fluctuations of temperature were very similar. He concluded, therefore, that neither thermoperiodicity nor irradiance could be considered differentiating factors, and that the two areas differed mainly in temperature and length of day, the temperature being higher and the days shorter in the southern area.

Of the factors indicated by Carder (2) only temperature appeared to be a suitable subject for investigation, because the longer photoperiod of the northern area would tend to hasten the development and maturity of flax (8). The effects of temperature were studied in conjunction with the effects of far-red radiation on which there is no information in flax. The effects of red and far-red radiation from incandescent lights on the photo regulation and elongation of many plants has been reported by Borthwick (1), Stolwyck (9), Downs (4), and others.

In the present paper the response of Raja and Redwing flax to variations in temperature and light in growth chambers is reported. These results are used to interpret the differences in behaviour of these varieties under natural environment in the southern and northern areas.

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MATERIALS AND METHODS

The experiments reported herein were carried out in two separate chambers having identical photoperiod (18 hours) and identical light intensity (1200 foot-candles at plant level). In one chamber, 4500°K. cool white fluorescent tubes, 75 watts each, were used exclusively; in the other, two of the fluorescent tubes were replaced by 60-watt standard G.E. tubular incandescent lamps, both light sources being used concurrently. The incandescent lights provide a considerable amount of radiant energy beyond 6500 Angstroms (10). Experiments with these light differentials were carried out at constant temperatures of 70° and 80°F.

The variety Raja was represented by nine foundation lines and Redwing by five selections. Raja is a fairly recent variety and slight differences were observed in field tests in the climatic reactions of these lines. In each experiment, each line, represented by two plants in a 5-inch clay pot, replicated five times. Several seeds were sown and seedlings were thinned to obtain the correct stand. Following germination the plants were watered once a day for 4 weeks, then twice a day until 2 weeks after blossoming, then once a day until there was no further growth. This rate of watering, subsequently referred to as "standard", was not sufficient for large plants at the 80°F. temperature. Experiments at that temperature were repeated with a higher watering rate sufficient to keep the soil in the pots moist constantly.

The onset of flowering and length of stem at that stage were recorded for each plant. The growth in height of each plant was recorded at 6-day intervals from germination to cessation of growth.

Growth of the branches was measured, beginning with the onset of flowering when they were only 1-2 centimetres long. Growth was considered to have ceased when the rate of elongation became less than 2 centimetres per 6-day interval. The time when this occurred was obtained by interpolation on the growth curve.

Field data for Raja and Redwing were obtained from the replicated yield tests conducted annually at 14 experimental stations in Canada (3).

EXPERIMENTAL RESULTS

The effects of the light and temperature treatments on the onset of flowering and on the stem and branch elongation will be considered separately. No significant differences were found between the lines of each variety. Therefore, the results of all lines of each variety were pooled.

Onset of Flowering

Supplementary red light and temperature both affected the onset of flowering of Raja and Redwing flax. Although incandescent light significantly advanced the flowering time of both varieties, its effect was much greater on Raja than on Redwing (Table 1). Without incandescent light, Redwing flowered 3.9 days earlier than Raja at 70°F. and only 2.3 or 5.5 days earlier at 80°F., depending on the water supply.

Flowering time of both varieties was also significantly advanced by high temperature but its effect was much greater on Redwing than on Raja (Table 1b). No change in trend was observed under the higher watering rate.

TABLE 1. — THE EFFECT OF TEMPERATURE AND INCANDESCENT LIGHT ON THE ONSET OF FLOWERING OF REDWING AND RAJA FLAX

Light source	Watering rate	Temperature (°F.)	Number of days to blossoming		Difference in days
			Redwing	Raja	
Fluorescent alone	Standard	70	71.3	75.2	−3.9*
	"	80	52.9	66.2	−13.3**
	High	80	51.2	67.9	−16.9**
Fluorescent plus incandescent	Standard	70	65.4	62.2	+3.2*
	"	80	50.1	52.4	−2.3*
	High	80	45.9	51.4	−5.5**

Difference in number of days to blossoming due to:

(a) Incandescent light

	Redwing	Raja
70°F. standard watering rate	−5.9**	−13.0**
80°F. " " "	−2.8*	−13.8**
80°F. high watering rate "	−5.3**	−16.5**

(b) Higher temperature

	Fluorescent alone		Fluorescent plus incandescent	
	Redwing	Raja	Redwing	Raja
Standard watering rate	−19.4**	−9.0**	−15.3**	−9.8**
High watering rate	−20.1**	−6.3**	−19.5**	−10.8**

* and ** mark significant difference at the 0.05 and 0.01 probability level, respectively.

Length of Stem

The lower watering rate used in the first high temperature experiments restricted the rate of elongation of the plants, although the plants showed no signs of wilting. The true effects of incandescent light and temperature can, therefore, be ascertained only from the second series of experiments in which a higher rate of watering was used.

It is quite evident that incandescent light caused a different response in the two varieties (Table 2a). It reduced the length of stem of Raja at both 70° and 80°F., and of Redwing at 80°F. alone and then only slightly (Table 2a). The effect of temperature varied depending on the presence or absence of incandescent light. Without it temperature caused a small increase in the height of Redwing but had no significant effect on Raja (Table 2b). However, under incandescent light the high temperature increased the stem length of Raja significantly but did not affect Redwing.

TABLE 2. — THE EFFECT OF TEMPERATURE AND INCANDESCENT LIGHT ON THE LENGTH OF STEM OF REDWING AND RAJA FLAX

Light source	Watering rate	Temperature (°F.)	Redwing	Raja	Difference (cm.)
Fluorescent alone	Standard	70	64.0	81.0	−17.0**
	"	80	49.6	59.1	−9.5**
	High	80	67.6	79.4	−11.8**
Fluorescent plus incandescent	Standard	70	64.3	59.6	+4.7*
	"	80	52.3	50.0	+2.3
	High	80	62.0	68.0	−6.0*

Difference in stem length due to:

(a) Incandescent light

	Redwing	Raja
70°F.	+0.3	−21.4**
80°F. (standard watering rate)	+2.7	−9.1**
80°F. (high watering rate)	−5.6	−11.4**

(b) High temperature

	Fluorescent		Fluorescent plus incandescent	
	Redwing	Raja	Redwing	Raja
Standard watering rate	−14.4**	−21.9**	−12.0**	−9.6**
High watering rate	+3.6**	−1.6	−2.3	+9.4**

* and ** mark significant difference at 0.05 and 0.01 probability level, respectively.

Duration of the Growth Period of Branches

The period of elongation of the branches of Redwing was reduced by 8-10 days under high temperature (80°F.) and that of Raja by 13-18 days (Table 3). The rate of watering apparently had no effect. Incandescent light caused a slight reduction in Redwing and none in Raja. The statistical significance of the differences between treatments could not be determined, because the data represent averages obtained by interpolation.

Length of Total Period of Growth

The greatest reduction in total period of growth was attained when both high temperature and incandescent light were available. The higher watering rate reduced the duration growth of Redwing but had no effect on Raja.

TABLE 3. — THE EFFECT OF TEMPERATURE AND SUPPLEMENTARY RED LIGHT ON THE LENGTH OF THE GROWTH PERIOD OF THE BRANCHES OF REDWING AND RAJA FLAX AND OF THE TOTAL GROWTH PERIOD OF THE PLANTS

Light source and watering rate	Temperature (°F.)	Duration of growth (days)			
		Branches		Plants	
		Redwing	Raja	Redwing	Raja
Fluorescent					
Standard watering rate	70	28	30	99.3	105.2
" " "	80	20	12	72.9	78.2
High " "	80	18	12	69.2	79.9
Fluorescent and incandescent					
Standard watering rate	70	24	26	89.4	88.2
" " "	80	15	13	65.1	65.4
High " "	80	15	13	60.9	64.4

Rate of Elongation

The rate of elongation has been presented in Figure 1 and Table 4 by the increments in height occurring at 6-day intervals. The rate increased from germination to shortly before the onset of flowering, when a gradual decrease set in. The rate of growth of both varieties was increased by higher temperature and by incandescent light in combination with high temperature. As the rate increased, the length of the growing period decreased. The rate of increase was greater for Redwing than for Raja, except under the combination of 70°F. and supplementary incandescent light.

Following blossoming the rate of elongation gradually diminished. Based on the average of three 6-day intervals of all treatments, the decrease in rate of growth was considerably greater for Redwing (46.0 per cent) than for Raja (38.2 per cent). Differences in rate of decrease due to treatments were small.

DISCUSSION

It is evident from this experiment that, in studying the effects of incandescent light and temperature on the development of flax, phasic development of the plants must be taken into consideration. In this experiment, the total period of growth was divided into two stages, one preceding and the other following the onset of flowering. The onset of flowering was chosen because it is a well defined and easily observable characteristic and coincides fairly closely with the end of stem elongation and the beginning of branch formation.

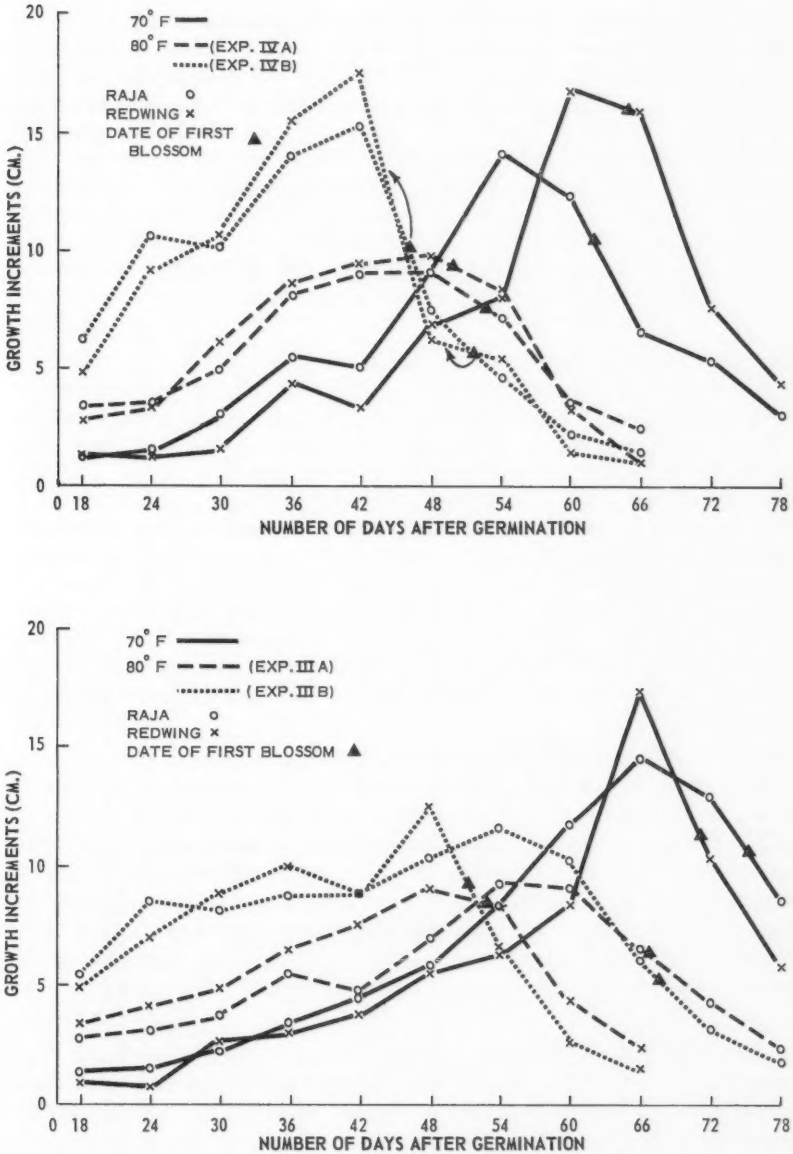

FIGURE 1. Height increments (cm.) at 6-day intervals of Raja and Redwing flax without incandescent light (*lower figure*) and with incandescent light (*upper figure*). A and B in Experiments III and IV refer to "standard" and "high" watering rate respectively.

TABLE 4. — INCREMENTS IN HEIGHT (CM.) OF RAJA AND REDWING PLANTS AT 6-DAY INTERVALS

Days after germination		Temp. °F.	18	24	30	36	42	48	54	60	66	72	78
Var.	Light source												
Raja													
	Fluorescent	70	1.6	1.6	2.3	3.5	4.6	5.9	8.6	11.9	14.7	13.1*	8.7
	Fluor. & incand.	70	1.3	1.7	3.1	5.6	5.1	9.2	14.2	12.4*	6.8	5.5	3.2
	Fluorescent (IIIA)	80	2.9	3.1	3.8	5.6	4.8	7.1	9.4	9.2	6.7*	4.5	2.5
	,, (IIIB)	80	5.5	8.8	8.3	8.9	9.0	10.5	11.9	10.4	6.2*	3.3	2.0
	Fluor. & incand. (IVA)	80	3.4	3.6	5.0	8.1	9.1	9.1*	7.2	3.8	2.7	–	–
	,, ,, (IVB)	80	6.2	10.8	10.2	14.0	15.4	7.6*	4.8	2.2	1.7	–	–
Redwing													
	Fluorescent	70	1.0	0.8	2.7	3.1	3.9	5.8	6.5	8.8	17.6*	10.5	6.0
	Fluor. & incand.	70	1.4	1.4	1.7	4.4	3.5	7.0	8.1	16.9*	16.0	7.8	4.5
	Fluorescent (IIIA)	80	3.5	4.2	4.9	6.7	7.7	9.1*	8.5	4.6	2.4	–	–
	,, (IIIB)	80	5.0	7.0	8.9	10.1	9.0	12.8*	6.7	2.7	1.6	–	–
	Fluor. & incand. (IVA)	80	2.9	4.6	6.1	8.5	9.3	9.9*	8.3	3.6	1.1	–	–
	,, ,, (IVB)	80	4.9	9.1	10.8	15.7	17.6*	6.4	5.5	1.6	1.2	–	–

*Marks the approximate time at which flowering occurred.
A and B in experiments III and IV mark experiment with standard and high watering rate.

The onset of flowering of both varieties was not affected by the slight differences in watering rates, but it was accelerated by incandescent light and high temperature (80°F.). The two varieties differ considerably in degree of response, Raja showing the greatest response to incandescent light, Redwing to temperature. The early blossoming induced in Redwing by high temperature can be equalled by Raja only when both high temperature and incandescent light are available. Field data show that the onset of flowering of both varieties occurs almost simultaneously in northern areas, but occurs from 3 to 6 days earlier for Raja in southern areas (3). The latter behaviour represents an important gain in maturity for Raja because the post-blossoming period of Raja is always longer than that of Redwing in both areas.

Only part of the period from the onset of flowering to maturity was studied under controlled conditions. This part, from the onset of blossoming to cessation of growth, corresponds closely to the blossoming period of flax, since blossoming continues to occur in conjunction with branch growth. When sufficient moisture is available, the length of the post-blossoming growth period is mainly restricted by high temperature, the restriction being considerably greater for Raja than for Redwing. Incandescent light has only a minor effect. Concurrent field data show that the length of this period can vary, between years, from 9 to 27 days for Redwing and from 11 to 33 days for Raja and that on the average this period is 2 to 3 days longer for Raja than for Redwing. It is evident from the data that Raja can equal Redwing in the length of this period if temperature is sufficiently high. This would occur more frequently in southern areas.

The only information available for the period from cessation of growth to maturity is derived from field experiments. The two varieties require from 3 to 6 weeks for this period and on the average Raja requires 2 to 4 days longer than Redwing, regardless of the area where it is grown. The length of this period appears to be controlled by moisture supply and temperature. However, these effects can be assessed to a limited degree only from interpretation of available field data. Part of the delay for Raja during this stage can be attributed to the fact that it has considerably larger seed than Redwing. Delayed maturity due to large seed was demonstrated by Plonka (7).

The above information points out that the length of the life cycle of Raja and Redwing flax, from germination to maturity, is controlled by their individual responses to radiant energy, temperature and moisture availability at different stages of development. The final result is a complex derivative.

The effects of incandescent light and temperature on total stem elongation and the rate at which elongation occurs are different and must be considered separately. High temperature (80°F.) does not necessarily restrict the height of different varieties, neither does incandescent light. However,

high temperature stimulates the rate of growth and incandescent light produces a similar effect but to a lesser extent. An increase in growth rate of fibre flax in growth cabinet was attributed by Millikan (6) to high temperature. Hansen and McGregor (5), on the contrary, reported a depressing effect in field experiments, but this must be ascribed to lack of moisture.

Following blossoming, the rate of growth declined more rapidly for Redwing than for Raja. High temperature slightly increased the rate of decline for Raja.

An examination of the growth curves in relation to the onset of flowering indicates that the period between peak growth and the onset of flowering varies for the two varieties. The exact time when flower initiation occurs has not been determined but, since the first bud becomes visible shortly after the peak is reached, it is possible to presume that flower initiation marks the end of maximum growth, that the period between it and the onset of flowering forms a separate phase within the growth cycle, and that the length of this period is subject to varietal differences. The exact point of differentiation is hard to determine and the onset of flowering presents the most practical reference point for the plant breeder.

The results obtained leave no doubt that varieties of flax differ in their response to light and temperature at various stages of growth and development. It is reasonable to assume that such differences are genetically controlled and that, consequently, it should be possible, under controlled conditions, to combine the most desirable of such responses into one variety. This, of course, is at present being attempted by plant breeders in a very haphazard manner and under impossible conditions, in view of the complete lack of information with regard to the phasic response of plants to particular environments, and the lack of control necessary to recognize these responses. It is evident that a systematic approach to plant breeding, based on these premises, would be extremely complex if all variables are taken into consideration. However, some of the main responses, demonstrated in the above experiment, might readily be combined into one variety.

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THE OCCURRENCE OF LOOSE SMUT RESISTANCE IN 42, 28 AND 14 CHROMOSOME WHEATS[1]

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[Received for publication May 11, 1961]

ABSTRACT

A total of 865 spring wheat varieties, and selections from the 14, 28 and 42 chromosome groups, were inoculated in 3 successive years with a mixture of Canadian collections of loose smut, *Ustilago tritici* (Pers.) Rostr. At the end of the first year, 231 were rated immune; after the second inoculation, 188; and after the third, 162. These included 116 from the 42 chromosome group, 43 from the 28, and 3 from the 14. Although some of the reduction in immune or resistant types after the first year may have resulted from escapes, it is probable that most of these showed susceptibility to new collections added in the second and third years.

INTRODUCTION

In the early part of this century losses caused by loose smut, *Ustilago tritici* (Pers.) Rostr., were quite appreciable in North America. Tapke (2) estimates that, in the period 1917-1926, annual losses from this disease were more than 10,000,000 bushels in the United States. No comparable figures are available for Western Canada. In recent years, however, loose smut has been relatively unimportant in wheat production in the Prairie Provinces. The principal varieties grown are Thatcher and Selkirk, which are immune and highly resistant, respectively. The only highly susceptible variety released in the last decade was Lee which, at its widest distribution in 1954, occupied only 20 per cent of the Manitoba and a smaller percentage of the Saskatchewan acreage.

Tapke (2) listed the reaction for 234 varieties and lines available to him in 1929. Of these, he found 13 which were immune. In 1957, observation rows of nearly 1,100 spring wheat varieties were grown in the field at Winnipeg in order to determine their possible value as plant breeding or genetic material. As many of the varieties as possible were inoculated to a mixture of loose smut collections to assess their resistance. In this paper are listed those varieties which remained immune after 3 years of inoculation.

MATERIALS AND METHODS

The wheat varieties grown in the 1957 genetic nursery were obtained from 28 countries of the world. One or two heads of 865 of the spring types were inoculated with a mixture of 26 collections of loose smut gathered throughout Canada in 1956.

Inoculation was effected by means of a Meyer's Silver Prince sprayer which was set to deliver the spore suspension at a pressure of 270 pounds per square inch (1) through a fine jet nozzle. In this method the wheat head is held in one hand while the spray is directed at the florets. The high pressure of the jet stream forces the glumes open, allowing the inoculum to enter the florets. The operation was carried out 1 or 2 days following anthesis of the central spikelets of selected heads.

[1]Contribution No. 68 of the Canada Department of Agriculture Research Station, Winnipeg, Man.

The inoculum was prepared by shaking equal numbers of smutted heads of each collection in a pail of water and straining the suspension into the sprayer tank through a screen of sufficiently large mesh to allow spores to pass through but to hold back rachis and floral parts. Enough water for a 4-hour supply was added. Fresh inoculum was prepared after 4 hours' use to ensure viability of the spores.

In 1958 the inoculated seed of each variety was sown in hills in a field nursery and the plants examined for smut infection. If even one head were smutted the variety was discarded. Where no infection occurred, one or two heads were again inoculated, this time with 26 collections of loose smut which had been collected in 1957. The inoculated seed was sown in 1959 and treated in a similar manner. The procedure was repeated in 1960 making a total of 3 successive years of testing.

RESULTS AND DISCUSSION

Of the 865 inoculated varieties grown in 1958, only 231 were rated immune. These were further reduced in 1959 to 188 and, after elimination in 1960, 162 remained. Of these, 116 were in the 42 chromosome group, 43 in the 28, and 3 in the 14. These varieties are grouped in Table 1 according to chromosome number. Where possible, sub-groups such as Thatcher and its derivatives are placed together. Certain agronomic and disease reaction data, recorded in 1957, are included to show the relative value of the varieties for characteristics other than loose smut resistance.

A. B. Campbell* has shown that Thatcher possesses a dominant gene for loose smut immunity. This is reflected in the immunity displayed by Thatcher and its derivatives. Other groups of spring wheat include some of the Kenya varieties and varieties with Supremo, Africa 43 or Maria Escobar as a common parent. None of the varieties listed in Table 1 was tested by Tapke (2).

A narrow genetic base was used for screening each variety. In the first year only one or two heads were inoculated and, since succeeding inoculations were made on plants grown from seed which had been inoculated, all tests were made on plant progenies tracing directly to these original heads. Thus, if the variety were heterogeneous for resistance, and an immune genotype were selected by chance, the variety would be considered immune. Heterogeneity for loose smut resistance may be more widespread than is usually realized. Unless wheat breeding programs are designed to incorporate loose smut resistance, no selection is made for this characteristic, and a variety may be released which carries both resistant and susceptible lines. In many tests for varietal reaction, some varieties are immune, others carry trace to 30 per cent smut, while still others are completely or nearly completely susceptible. The question is whether all those in the trace-to-30 per cent group are susceptible, or whether only a portion of the population is susceptible. Physiologic specialization in *Ustilago tritici* may provide a second alternative. This organism passes through a sexual cycle in each generation and as a result many races may be present. Thus, in those cases where 80 to 100 per cent infection is observed,

*Personal communication

TABLE 1. — AGRONOMIC AND DISEASE DATA FOR 1957 ON VARIETIES AND SELECTIONS OF SPRING WHEAT IMMUNE TO LOOSE SMUT FOR A 3-YEAR PERIOD

Winnipeg Genetic No.* WG 57-	Origin of seed	Days to head	Days to mature	Leaf rust %	Stem rust %	Lodging resistance 1-9	Awnedness	Black chaff 1-9
42 Chromosomes								
09	USA	65	106	T	T	4	Ad	1
10	,,	66	106	T	0-20	6	Ad	1
11	,,	64	99	0	0	4	Ad	2
15	,,	64	98	0	T	4	Ad	3
17	,,	66	106	T	3	4	Ad	1
358	,,	62	96	60	5	2	Ad	3
356	,,	62	97	70	15	2	Al	2
1063	Canada	62	94	60	60	3	Al	4
203	Germany	63	99	50	40	2	Al	2
1070	USSR	57	91	80	10	2	Al	5
676	USA	66	100	50	30	3	Ad	6
982	Canada	64	99	50	10	3	Al	6
961	,,	63	97	40	1	1	Al	7
984	,,	63	100	T	1	3	Ad	3
310	USA	66	99	25	20	1	Al	3
1050	,,	59	94	50	40	2	Al	2
377	,,	63	97	50	10	2	Al	4
742	Mexico	64	98	5	0	4	Ad	6
741	,,	64	100	20	0	3	Ad	4
727	,,	60	97	0	T	3	Ad	5
726	,,	62	96	5	0	5	Ad	8
81	,,	61	97	15	2	2	Ad	1
942	USA	63	100	T	1	3	Ad	7
752	Mexico	63	101	5-10	0	2	Ad	8
206	Kenya	65	99	60	20	3	Al	2
209	,,	64	102	50	60	2	Al	3
871	,,	65	100	40	0	2	Al	3
18	,,	69	101	3-5	5	6	Al	1
873	,,	75	104	5	30	3	Al	2
224	,,	65	103	60	30	6	Al	2
1085	,,	58	90	20	80	3	Al	4
97	,,	62	98	80	1	2	Al	2
96	,,	65	99	70	1	2	Al	3
22	,,	66	105	15	40	2	Al	2
23	,,	64	98	10-50	0	5	Al	2
897	,,	65	103	5	15	2	Al	3
899	,,	72	100	15	40	1	Al	2
901	,,	63	99	80	1	2	Al	6
650	,,	65	106	80	30	2	Al	2
935	Mexico	65	103	1	20	4	Ad	2
49	,,	62	97	0	T	2	Ad	3
139	,,	66	103	5	0	3	Al	2
148	Mexico	60	91	0	0	1	Al	6
733	,,	59	97	0	0	3	Ad	6
123	,,	64	99	0-60	T	1	Al	2
721	,,	61	99	40	T	2	Al	4
746	,,	65	97	0	0	5	Ad	3
70	,,	64	102	0	20	2	Al	3
152	,,	63	98	T	0	2	Ad	3
734	,,	63	99	0	0	4	Ad	4
74	S. Africa	63	97	0	5	2	Al	3
158	Mexico	61	94	0	0	2	Al	9
921	S. Africa	64	103	0	40	3	Al	6
922	,,	63	102	0	30	3	Al	4
923	,,	65	100	0	30	2	Al	6

TABLE 1. — *continued*

Winnipeg Genetic No.* WG 57-	Origin of seed	Days to head	Days to mature	Leaf rust %	Stem rust %	Lodging resist-ance 1-9	Awned-ness	Black chaff 1-9
42 Chromosomes								
924	S. Africa	65	100	0	25	3	Al	5
925	,,	64	102	0	40	1	Al	4
926	,,	64	102	0	30	1	Al	3
927	,,	65	103	0	40	1	Al	4
928	,,	65	103	0	25	2	Al	4
929	,,	65	103	0	10	1	Al	2
930	,,	65	98	0	5	2	Al	3
931	,,	66	104	0	20	2	Al	2
932	,,	65	103	0	10	5	Al	3
933	,,	62	95	50	1	6	Ad	7
107	S. Africa	64	98	0	5	1	Ad	2
1076	USA	59	93	50	5	5	Ad	5
939	Peru	62	99	0	15	3	Ad	3
94	,,	63	96	T	0	1	Ad	4
801	,,	64	99	T	0	2	Ad	7
802	,,	63	98	1	0	2	Ad	8
1072	USA	59	94	60	10	4	Ad	2
835	Colombia	63	97	T	0	4	Al	6
111	Argentina	65	101	5	0	4	Ad	3
214	,,	65	98	0	80	3	Ad	2
233	,,	65	99	T	70	4	Ad	2
704	,,	63	98	0	T	5	Ad	8
245	USA	66	101	40	5	4	Al	4
674	,,	61	99	30	5	3	Al	8
675	,,	65	97	50	20	4	Al	4
1005	Canada	58	92	10	5	3	Seg.	5
682	USA	60	104	5	0	1	Ad	2
776	,,	60	103	1	0	2	Ad	4
778	,,	61	104	5	0	3	Al	5
673	,,	61	107	40	0	7	Ad	3
340	,,	64	99	80	50	5	Ad	4
347	,,	64	99	70	T	3	Ad	2
350	,,	63	97	40	90	3	Ad	6
646	,,	63	99	50	40	2	Ad	2
545	Italy	62	96	5	90	1	Ad	4
661	,,	64	95	0	50	2	Seg.	4
519	Switzerland	63	95	30-90	100	2	Ad	2
522	,,	64	97	30	80	1	Seg.	3
517	,,	64	98	50	50	1	Seg.	4
518	,,	64	98	30	40	1	Seg.	2
630	Canada	61	97	70	40	2	Ad	2
821	Chile	65	102	30	0	2	Al	6
823	,,	61	99	1	0	3	Al	8
824	,,	62	107	1	0	2	Ad	3
825	,,	61	106	T	0	2	Ad	6
822	,,	61	99	10	0	3	Al	3
830	Colombia	71	107	5	25	2	Al	4
831	,,	61	95	3	5	3	Al	7
832	,,	64	98	60	T	5	Al	8
1024	Australia	60	88	100	100	2	Al	5
183	,,	61	92	90	100	2	Al	3
443	Canada	62	93	60	90	1	Al	2
422	,,	68	96	80	90	2	Al	2
625	Eire	68	99	40	90	1	Al	2
87	Canada	60	97	10-80	70	1	Al	2
1054	USA	59	88	10	100	3	Ad	2

TABLE 1. — *continued*

Winnipeg Genetic No.* WG 57-	Origin of seed	Days to head	Days to mature	Leaf rust %	Stem rust %	Lodging resist-ance 1-9	Awned-ness	Black chaff 1-9
42 Chromosomes								
758	Mexico	60	97	15	0	3	Al	8
1143	Czechoslovakia	55	84	100	100	3	Ad	8
498	Canada	67	97	30	100	3	Ad	2
1110	Czechoslovakia	62	94	30	100	3	Ad	3
490	Canada	70	110	15	90	3	Ad	2
28 Chromosomes								
343	Australia	64	97	0	30	4	Ad	3
344	,,	64	95	0	T	4	Ad	3
387	Italy	62	97	0	T	5	Ad	3
391	,,	62	97	0	15	4	Ad	3
683	USA	64	100	0	0	8	Ad	2
685	,,	67	106	0	5	7	Ad	5
690	,,	63	101	0	1	5	Ad	3
810	,,	63	94	0	0	7	Ad	3
811	,,	62	95	0	15	6	Ad	4
812	,,	64	95	0	0	8	Ad	5
816	,,	64	99	0	0	6	Ad	3
808	,,	61	95	0	0	6	Ad	5
807	Canada	68	95	0	2	5	Ad	3
688	,,	68	107	0	30	9	Al	5
817	Tunis	65	99	0	0	5	Ad	5
839	Portugal	68	106	0	40	7	Ad	2
944	,,	62	90	5	0	6	Ad	7
691	Ethiopia	62	99	T	0	6	Ad	4
945	,,	62	94	1	0	7	Ad	3
947	,,	63	95	T	0	5	Ad	6
951	,,	65	96	5	T	5	Ad	4
949	Spain	65	95	0	0	4	Ad	4
950	France	66	97	0	T	6	Ad	5
952	Arabia	63	94	0	0	5	Ad	4
1152	Cyprus	60	95	T-5	–	4	Ad	5
703	Egypt	62	95	0	T	9	Ad	7
304	Canada	60	94	T	0	2	Ad	3
312	,,	73	102	5	10	3	Ad	2
482	,,	70	100	T	40	4	Ad	2
483	,,	69	100	2	90	4	Ad	2
485	,,	69	100	0	60	2	Ad	4
1121	Czechoslovakia	66	100	5	100	3	Ad	3
1125	,,	66	95	T	80	6	Ad	2
1128	,,	68	102	0	80	3	Ad	–
1126	,,	62	94	0	90	5	Ad	2
486	Canada	66	95	40	100	4	Ad	2
1017	USA	62	92	80	50	5	Ad	3
837	Canada	68	102	0	0	6	Ad	2
1049	,,	60	93	0	40	4	Ad	1
1051	,,	59	93	2	40	4	Ad	3
445	,,	75	96	5	90	2	Ad	2
1095	Czechoslovakia	62	93	0	20	4	Ad	2
1096	,,	61	95	0	70	3	Ad	3
14 Chromosomes								
1073	USA	59	94	0	0	6	Ad	1
447	Canada	69	107	0	1	4	Ad	1
694	USA	65	100	T	60	8	Ad	2

*Variety or selection names

42 Chromosomes

09 Frontana-Thatcher
10 ,,
11 ,,
15 ,,
17 ,,
358 H-44 x Thatcher
356 Thatcher
1063 Thatcher selection
203 Hochzucht (similar to Thatcher)
1070 Kostoff's triple hybrid (sim. to That.)
676 Pilot^2 x Thatcher
982 Thatcher x Regent
961 Thatcher^6 x Kenya Farmer
927 Red Egyptian Type 170918
928 ,, 170918-2c
929 ,, 170919
930 ,, 170919-1c
931 ,, 170922
932 ,, 170922-1c
933 ,, 170923
107 ,, 170925
1076 H-44-24
939 Maria Escobar
94 Maria Escobar x H-44-Marquis
801 ,,
802 ,,
984 (Canus x Thatcher) x (482.B x Regent)
310 Double Cross 825-4
1050 Marquillo x Waratah
377 Newthatch
742 (Mentana x Merit-Kenya) x (Kenya-Gular-Pilot) - (Kenya 58-Newthatch)
741 Merit (Newthatch-Marroqui) Kentana x (Kenya-Gular-Pilot) x (Kenya 58-Newthatch)
727 (Kenya-Redman-Dundee) x Newthatch
726 Candeal-Newthatch-Kentana
81 (Kenya-Gular-Pilot) x (Kenya 58-Newthatch)
942 Willet
752 Willet x Lerma
206 Kenya I-40-34
209 ,, I-40-45
871 ,, I-49-157
18 ,, 117A
873 ,, 117A I-49-161
224 ,, 235.A.1.A.4
1085 ,, B.256.G
97 B.286
96 ,, 294.B.2.A.3
22 ,, 321.AD.1.C.3
23 ,, 321.BT.1.B.1
897 ,, 338.Z.2.G.2
899 ,, 340.Y.4.A.1
901 ,, 340.Y.6.B.3
650 ,, 351.AS.1.B.2
935 Supremo
49 Peru x Supremo
139 Supremo-Kenya x Egypt-Timstein
148 Supremo-Timstein-Kenya
733 Supremo 211 x Lerma
123 Supremo^3 x Kenya
721 Kenya-Supremo x Yaqui
746 Wisc. 245 x Supremo 51

42 Chromosomes

70 (Mayo x Peru-Supremo) x (Peru-Kenya)
152 Mayo (Pelon, Colorado-Renown^2-Supremo) Kentana^2
734 ,,
74 Africa 43
158 Africa 43 x Yaqui 50
921 Red Egyptian Type 170915
922 ,, 170915-3c
923 ,, 170916
924 ,, 170916-2c
925 ,, 170917
926 ,, 170917-2c
1072 (Ceres-Hope) x Florence
835 Riccio x Yaqui-Kentana
111 Argentina No. 49-4824
214 Klein 40
233 Sinvalocho M.A.
704 Magnif disro
245 Mida-Cadet
674 Lee x 1831
675 Rescue x 1831
1005 (Mida-Cadet) x (McMurachy-Exchange x Redman), R.L. 2667
682 K 338 AA x Ns 3880-191, ND 52
776 ,, ND 57
778 ,, ND 59
673 Lee-Frontana II-47-10
340 Rival
347 Mercury
350 Premier
646 Russell
545 Oleata Calandria
661 Leone
519 B 1
522 B 15
517 263-25-2
518 263-27-2
630 C.D. 3872-4. C.A.N. 3740
821 Lleafen
823 Rosella
824 76 R
825 22 R
822 Redondo Negro
830 Frontana-Egypt Na 95
831 (Mida-Kenya 117 A) Frontana
832 Salles-McMurachy x Mayo 48
1024 Bunyip
183 Bobs
443 Red Bobs
422 Renfrew
625 Rosa Karn x Karn II
87 Cailloux
1054 Chinese x Webster
758 (Timstein-Kenya 58 x Gabo) Lee
1143 *T. vulgare* Vill.
498 *T. aestivum* L. var. ferrugineum
1110 *T. compactum* Host.
490 *T. spelta* L. var. coeruleum

28 Chromosomes

343 Gaza
344 ,,
387 Patrizio 6
391 Capeiti 8
683 Yuma, Ld. 364
685 Towner, Ld. 370

28 Chromosomes

690 Sentry
810 C.I. 3255 x Khapli-D.C. 50
811 ,,
812 ,,
816 Khapli-D.C. x Carleton 50
808 Ld. 340-Khapli x P.I. 94701
807 Golden Ball-Iumillo-Mindum, R.L. 1714
688 R.L. 3206
817 Medeah AP 9
839 Tremes preto
944 Amarai bianco tipo
691 St. 464
945 C.I. 7805
947 C.I. 7875
951 Camadi
949 Rojal de Almeria
950 Tounse
952 Arabian
1152 Giza
703 Beladi 116
304 Khapli
312 Black Persian
482 *T. durum* var. affine
483 *T. durum* var. hordeiforme
485 *T. durum* var. leucomelan
1121 *T. turgidum* L. 0-10-868
1125 *T. turgidum* L. 0-10-769
1128 *T. turgidum* L. 0-10-751
1126 *T. turgidum* L. 0-10-824
486 *T. turgidum* L. var. gentile
1017 *T. dicoccoides* I-38-55
837 Yaroslav emmer
1049 Emmer R.L. 116
1051 Early emmer R.L. 114
445 *T. polonicum* var. levissimum
1095 *T. pyramidale* 0-10-911
1096 *T. pyramidale* 0-10-920

14 Chromosomes

1073 *T. aegilopoides* I-38-21
447 *T. monococcum* var. hornemanii
694 Einkorn C.I. 2433

the variety may be susceptible to many biotypes, but where a low level is observed the variety may be susceptible to only a few of these: only those seeds infected by a virulent biotype give rise to smutted plants. New biotypes, therefore, may explain the sudden susceptibility of a number of immune varieties observed in the present investigation, since some new collections were included in the mixture each year.

Inoculation by means of a high pressure jet sprayer can be considered quite effective as many of the more susceptible varieties carried up to 100 per cent infection in the first year of testing. However, more refined tests using needle inoculatior might possibly reveal some susceptibility in the varieties which were retained. Such a procedure should be followed before the varieties reported herein as immune are used in a program breeding for resistance or in genetic studies for inheritance of resistance. Subject to these qualifications, it is considered that these varieties will be of value in the plant breeding and genetic fields.

ACKNOWLEDGEMENTS

The author is indebted to W. J. Cherewick for supplying inoculum of the different collections of loose smut, and to J. R. Murray who assisted in the inoculations.

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THE CONTROL OF GROWTH AND DEVELOPMENT IN RED CLOVER (*TRIFOLIUM PRATENSE* L.)

IV. VEGETATIVE PROPAGATION AND THE USE OF GROWTH REGULATORS[1, 2]

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[Received for publication November 24, 1960]

ABSTRACT

Improved methods for vegetative propagation of red clover are described. "Leaf-bud" propagules, consisting of a single trifoliate leaf with its stipules and a small axillary bud in a stage of indeterminate growth, provide better units for vegetative propagation than crown or stem cuttings and have shown a closer similarity to seedlings in their growth and development. All types of propagules can be rooted in full daylight by using an overhead mist-spray system with vermiculite as the rooting medium.

Propagule production can be very significantly increased by pre-treating intact plants with short daylengths and/or 2,3,5-triiodobenzoic acid (TIBA). Application of maleic hydrazide (MH) to flowering stems causes abortion of floral apices, thereby hastening and very significantly increasing propagule production. Auxin treatment of propagules and/or long daylengths during rooting, increase the per cent rooting and the amount of root formation.

Propagation in this manner can be very rapid. For example, more than 1,000 rooted propagules were obtained at one time from single field-planted propagules of a late-flowering clone in the first year. Propagules that were closely planted in wooden flats in vermiculite were maintained satisfactorily as very small plants overwinter under greenhouse and also under cold storage conditions, and were utilized in clonal-row field plantings for seed production.

INTRODUCTION

In agronomy and plant breeding, there is a particular need for efficient methods of vegetative propagation to provide uniform clonal material for experimental work and breeding programs. If first generation hybrid or synthetic seed is to be produced from clonal material of heterozygous leguminous plants such as alfalfa (16, 17) or red clover, large-scale vegetative propagation will be essential.

The present paper provides an assessment of the following main problems and objectives in the vegetative propagation of red clover:

1) — Can an overhead mist-spray system (7, 8, 13) provide a high per cent rooting under a wide range of conditions for all sizes and types of propagules?[5]

2) — Which aerial portions of the plant have the greatest potentiality for rooting, provide the most satisfactory types of propagules, with the most satisfactory growth and development when grown on? It is known that genotype and the stage of growth and development of red clover plants may influence subsequent rooting of propagules (1, 5). Investigators have generally found that cuttings obtained from the crown or the lower parts of the primary stem rooted more readily than those from the distal parts of the stem. These cuttings subsequently showed better crown growth (11, 15).

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[1]Based on part of a Ph.D. thesis accepted by the Faculty of Graduate Studies and Research, McGill University, Montreal, Que. 1956.

[2]Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Journal Series No. 484.

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[4]Professor of Agronomy, Macdonald College, Que.

[5]*Propagule*: Any unit for vegetative propagation capable of producing a mature plant with seed.

3) – What treatments should be applied to a plant for the maximum production of propagules? Endogenous auxin level, correlated with differences in root formation, has been shown to vary according to the genotype of the plant, also, according to the daylength and light intensity to which parental plants or propagules are exposed (5, 6). Tissue sensitivity, as expressed by root formation on propagules in response to a given level of applied auxin, may be influenced by relatively short-term daylength pretreatments of plants (5). Short daylengths will increase the amount of crown growth on plants and thus the proportion of propagules that will root readily (1). Some growth regulators are known to cause adventitious root formation on the aerial parts of intact plants (20). Growth regulators such as 3-indoleacetic acid (IAA), 2,3,5-triiodobenzoic acid (TIBA), and maleic hydrazide (MH) can significantly increase the amount of lateral bud formation on red clover plants under both greenhouse and field conditions (4, 5); this suggests a possible method of increasing propagule formation.

4) – Will direct treatments of propagules increase the amount of rooting? It is known that auxin treatment of propagules may increase rooting (5, 10, 12). Also, long daylengths as compared with short daylengths during rooting can stimulate root formation by increasing the endogenous auxin level (5, 6).

5) – Can large numbers of propagules be stored over winter in the greenhouse or under cold storage conditions without excessive requirements of space or labour?

MATERIALS AND METHODS

All studies were conducted with clonal material derived from the breeding nursery of the Dollard variety of red clover at Macdonald College, McGill University. The growth type classification of this variety has been discussed elsewhere (3, 4, 14). The methods and basis for selection of plants used in these studies, and, the methods for treatment of plants with growth regulating substances, have been referred to in the previous papers of this series (4, 5, 6).

Receptacles for Rooting Propagules

Two types of receptacles were used for rooting the propagules:

1) *Propagation frames* – 3 feet x 1½ feet x 1 foot capacity, made of 1-inch softwood. Removable glass sash 3 feet x 1½ feet that could be used to maintain a high humidity. Drainage by ¼-inch slits between the bottom boards as well as ¼-inch drilled holes. Used in rooting experiments when the mist-spray system was not employed. In these experiments, wilting of propagules was reduced as much as possible by judicious shading and watering.

2) *Standard flats* – 1 foot 4½ inches x 1 foot 2 inches x 3 feet capacity, made of ¾-inch softwood. Drainage by ¼-inch slits between the bottom boards. Used in experiments when a mist-spray system was employed.

Environmental Conditions for the Optimum Rooting of Propagules

Unless otherwise stated under "Experimental Results", an overhead mist-spray system (7, 8, 13) was used as a standard method for rooting propagules. Nozzles that provided a very fine mist, in the form of a flat

arc at a relatively low pressure, were used.* The nozzles were connected to a hose-line in the greenhouse and the water supply was controlled manually. Medium-coarse horticultural grade vermiculite was used as the rooting medium because it provided better rooting as compared with sand. Small propagules were introduced into the vermiculite with the aid of a bent blunt mounted needle. Preliminary experiments with 4896 stem cuttings indicated that, on the average, a 60 per cent increase in rooting could be obtained by the use of an overhead mist-spray under conditions when the air temperature rose to about 38°C. in full sunlight in the greenhouse.

Supplemental Illumination

In experiments involving the rooting of propagules under different daylengths, 300-watt reflector floodlamps provided supplemental illumination of 400 foot-candles at the level of the propagules. The mist-spray system enabled high humidities to be maintained while keeping temperatures between *ca.* 16 and 20°C. Propagules were exposed to full daylight whenever a mist-spray system was used.

EXPERIMENTAL RESULTS

Initiation of Roots on the Aerial Parts of Intact Plants

Tumescent growth and rootlet formation were induced after application of either IAA or NAA at concentrations of 10 mg./ml. 50 per cent ethyl alcohol. This occurred on leaflets, petioles, stipules, the nodal and internodal regions of stems, and sometimes upon the rachis of the inflorescence head. Figure 1 (A and B) illustrates the tumescent type of growth that occurred on young actively growing stems of plants of clone 339 after spraying with 10 milligrams NAA/ml. 50 per cent ethyl alcohol. This response was produced under greenhouse conditions in February; control stems showed no signs of tumescence or rootlet formation. Figure 1 (C and D) also illustrates the effects of treating stems and petioles of clone 7 with 2 milligrams IAA/gm. lanolin. In this experiment, as soon as tumescent growth became apparent on the IAA-treated tissues, stem and leaf portions were taken from the control and treated plants and were placed in water. Roots were rapidly initiated on the IAA-treated tissues and grew profusely.

Formation of rootlets upon intact stems may occur naturally at a certain stage of growth. Figure 2A illustrates a portion of a primary stem of clone 9 with lateral buds in the second cycle of vegetative growth. This stage of growth provided ideal units ("leaf-bud" propagules) for vegetative propagation; it occurred on plants that were grown under 16-hour long daylengths (LD) and then transferred to 12-hour short daylengths (SD) after initiation of stem elongation and visible floral formation. Details of this type of growth are shown in Figure 2B: at the tip of the contracted lateral shoot an aborted inflorescence (x) is present; below this, a lateral bud (y) of the second cycle of vegetative growth has two root primordia (z) present at the base of the bud below the stipular region of the subtending leaf. When plants with this type of growth were placed under conditions

*Teejet Nozzle No. 73003a, John Brooks Co. Ltd., Montreal, Que.

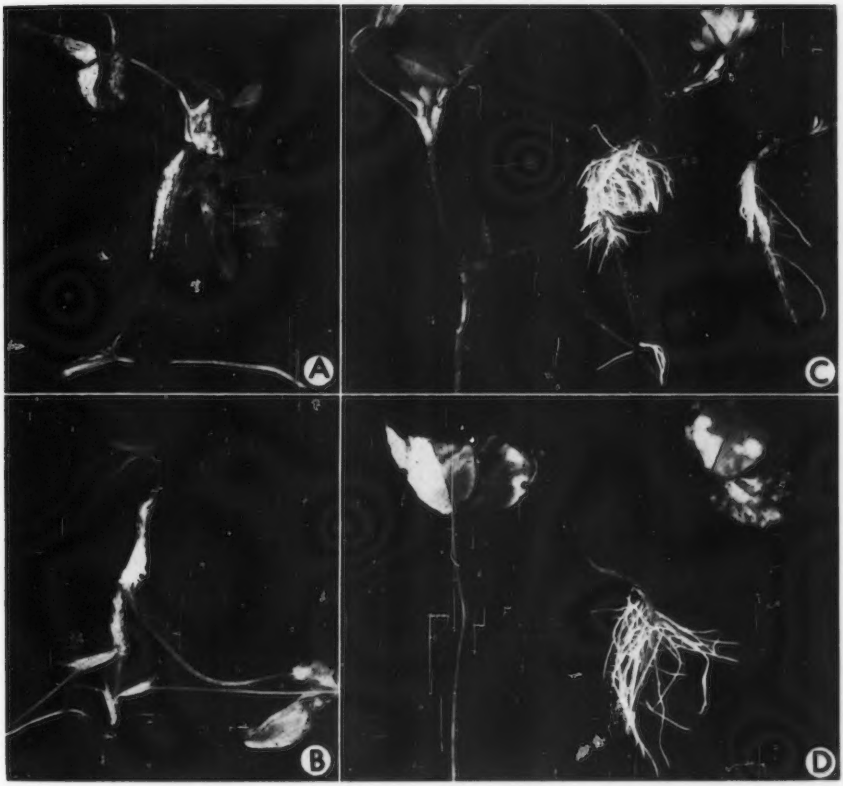

FIGURE 1. A and B. Young stem tissues of intact plan of clone 339 sprayed with 10 milligrams IAA/ml. 50 per cent ethyl alcohol; tumescent growth and formation of root primordia (A) and rootlet formation (B). C and D. Root formation on stems (C) petiole (D) after treatment of intact plant with 2 milligrams IAA/gm. lanolin; tissues placed for rooting in water, after tumescent response. Control portions of stem and leaf treated only with lanolin, shown on *left* in C and D.

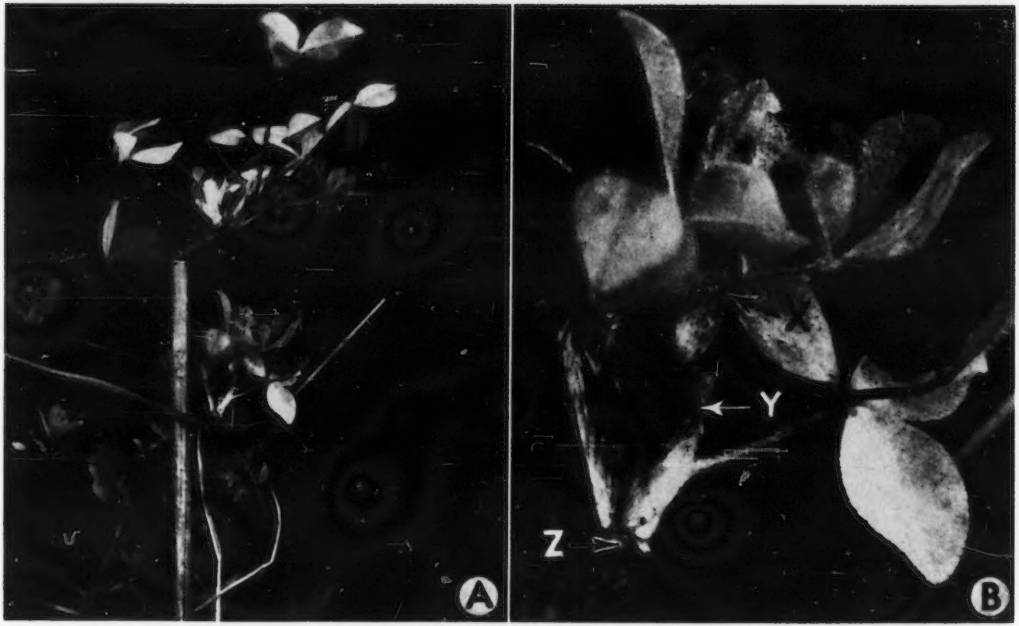

FIGURE 2. A. Lateral buds in the second cycle of vegetative growth, borne at nodes of first cycle stem. Clone 9. B. Details of lateral bud and shoot growth. Aborted inflorescence at tip of contracted lateral shoot (x), lateral bud in second cycle of vegetative growth (y) with two root primordia (z) at the base of the stipules.

of high humidity, rootlets were initiated from the root primordia. Application of a solution of 2 milligrams IAA/ml. 50 per cent ethyl alcohol to root primordia, of the type shown in Figure 2, resulted in considerable tumescent growth and the formation of numerous rootlets at the basal region of the lateral buds.

Types of Propagules

To clarify the differences that may occur according to the stage of growth and development of a red clover plant, the types of propagules that can be obtained are classified as (a) upper cuttings; (b) middle cuttings; (c) basal cuttings; (d) crown cuttings; (e) leaf-bud propagules. Propagules were prepared with the aid of a small pointed scalpel.

(a) *Upper cuttings* (Figure 3A: UC). Taken from the distal portions of a flowering stem usually with a single leaf and node. The stem portion was generally more succulent than on the basal cuttings (c). Rooting occurred quite readily on the lower cut surface of the stem. Because buds cannot be produced from the internodes, these cuttings only survived if axillary buds grew from a node and produced roots.

(b) *Middle cuttings.* Intermediate between the extreme types of upper and basal stem cuttings.

(c) *Basal cuttings* (Figure 3A: BC). Taken from the lower stem portions of the plant, usually with a single leaf and node. If the stem was still succulent, rooting occurred on all the peripheral regions of the basal cut surface – although most abundantly in the region directly below the lateral bud (Figure 3). If the stem was woody, root formation was restricted mainly to the area of leaf insertion.

(d) *Crown cuttings* (Figure 3B). Taken from the crown, usually with one or more primary and/or lateral buds in the first cycle of vegetative growth, with or without internode elongation. Crown cuttings may be equivalent to "leaf-bud" propagules [viz. (e)].

(e) *Leaf-bud propagules* (Figure 3A: LB). The small axillary buds of the second cycle of vegetative growth provided ideal units for vegetative propagation. The group of axillary buds illustrated in Figure 2 were detached simply by cutting them from the stem; it was then possible to obtain about 40 units for vegetative propagation ("leaf-bud" propagules) by isolating a single trifoliate leaf, petiole and stipules, with its small axillary bud situated at the base of the midrib on its inner surface. When leaflets and petiole only were taken, there was often rooting from the petiole but never any bud formation (Figure 3A: P).

Leaf-bud propagules were obtained from the first cycle of growth of a primary bud – from the crown tissues, but necessarily before appreciable stem elongation had occurred. However, these units were less readily isolated than those from second cycle vegetative growth and, depending on previous photoperiodic conditions, the buds sometimes possessed floral but no vegetative primordia.

When small leaf-bud propagules were taken, a single primary root was often formed, which eventually corresponded more closely to the tap root of seedlings. On the larger propagules, particularly stem cuttings, root initiation often resulted in the formation of numerous roots rather than a tap root (Figure 3).

Treatment of Plants with Growth Regulators Before Taking Propagules, and the Influence of Parental Growth Type

The following experiments were designed to test whether treatment of plants with growth regulators could increase the number of rooted propagules obtainable. Because either the crown, or stems in the second cycle of vegetative growth, provided the most suitable units for vegetative propagation, treatments were sought that favoured either of these stages under greenhouse or field conditions.

(i) *Treatment of plants with IAA and TIBA under greenhouse conditions*

In the following experiments, 16 plants of each of clones 13, 9, 12, and 23 (growth types 0, I, III and IV, respectively), grown in 4-inch pots, were transferred to continuous light. Eight plants of each clone were sprayed once a week for a total of four times with 1 milligram IAA per millilitre of lanolin emulsion (18) during October, 1954. On December 1, the plants possessed fully elongated stems and were transferred to natural daylength. Under these conditions, half the number of control and IAA-pre-treated plants were watered with TIBA, 200 p.p.m. on December 14, 1954, and 50 p.p.m. on December 20, 1954, and thereafter with 25 p.p.m. at intervals of 5 days. As many propagules as possible were taken on February 4, 1955, from the crown and stems of one plant of each clone chosen at random from each treatment. These propagules were primarily "leaf-bud" units and were placed for rooting in vermiculite under 16-hour LD using the mist-spray system.

TABLE 1. — PLANTS SPRAYED WITH 0 OR 1 MILLIGRAM IAA/ML. LANOLIN EMULSION UNDER CONTINUOUS LIGHT, THEN CONTROL AND TIBA TREATMENTS UNDER NATURAL DAYLENGTH. NUMBER OF PROPAGULES OBTAINED AND PER CENT ROOTED UNDER 16-HOUR DAYLENGTHS

Clone	Growth type	Lanolin emulsion				IAA/Lanolin emulsion				Mean	
		No. obtained		Per cent rooted		No. obtained		Per cent rooted		No. obtained	Per cent rooted
		0	TIBA	0	TIBA	0	TIBA	0	TIBA		
13	0	108	188	94.6	94.2	83	214	92.9	84.4	148.3	91.5
9	I	138	238	96.3	88.8	58	268	92.5	96.7	175.5	93.6
12	III	64	79	100	95.3	36	126	96.9	95.3	76.2	96.9
23	IV	76	194	87.5	86.7	47	123	87.5	93.8	110.0	88.9
Mean		96.5	174.8	94.6	91.8	56.0	182.8	92.5	92.6	127.5	92.7

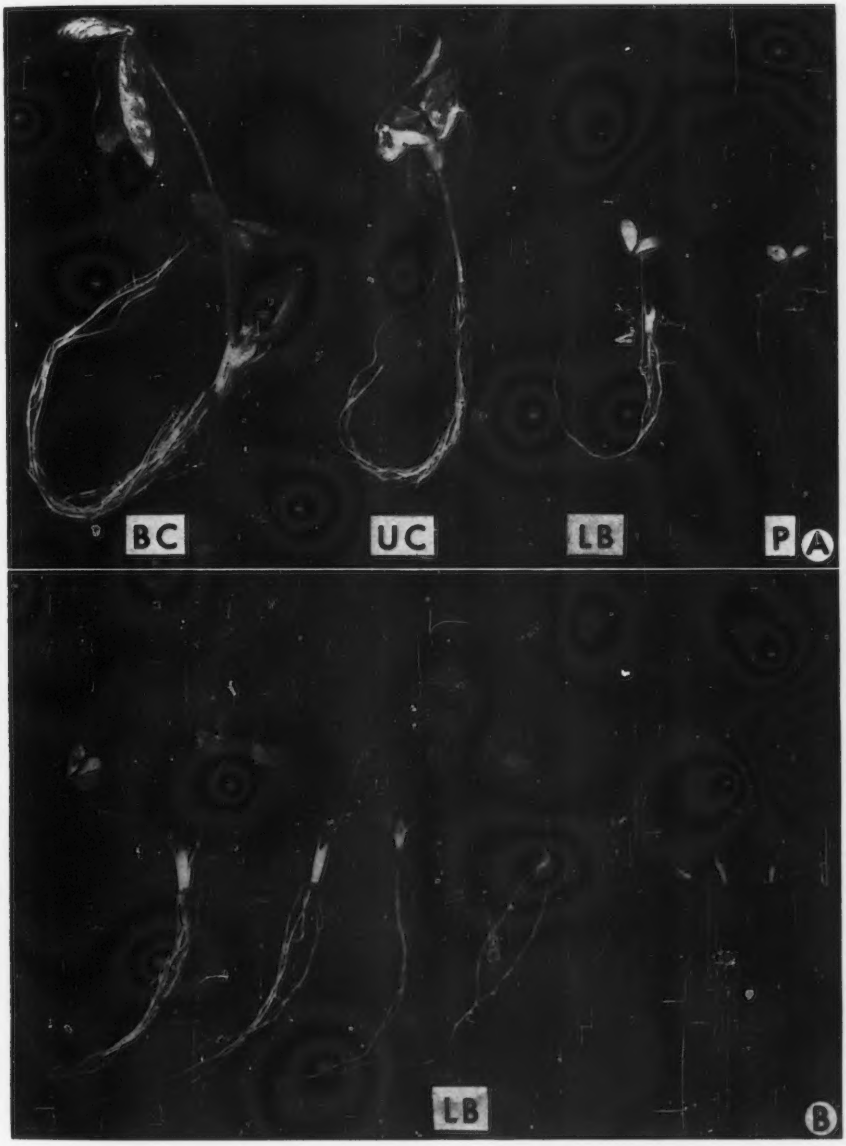

FIGURE 3. Different types of propagules obtained from red clover, clone 9; after rooting in vermiculite under mist-spray system. A. BC: basal cutting, UC: upper cutting, LB: leaf-bud, P: petiole. B. LB: the range in size of leaf-buds, those on *left* are comparable with crown buds or cuttings. *Note*: Numerous rootlets formed on larger propagules, fewer roots on smaller propagules which may form tap roots.

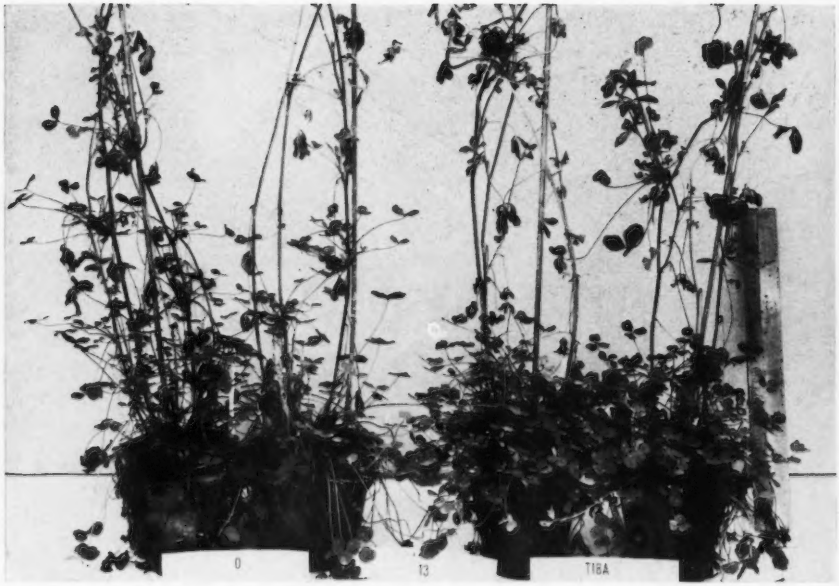

FIGURE 4. Bud and leaf growth on plants of clone 13 after watering soil with TIBA; control plants on *left*, TIBA-treated plants on *right*. Plants transferred from 16-hour to natur.l daylengths on December 3, 1954. (Pho:ographed February 2, 1955.)

Table 1 records the number of propagules obtained from each plant, and the per cent rooting from a representative sample of approximately 100 propagules of each clone. An increase in lateral bud formation, particularly on the crown, was obtained after control plants were treated with TIBA (Figure 4). Pre-treatment of the plants had no significant effect on the per cent rooting of propagules. At least 84 per cent rooting was obtained with all clones. The pre-treatments can, therefore, be evaluated directly for any effect in increasing the number of propagules that were obtained. The analysis of variance for number of propagules obtained, according to pre-treatment of the plants, was based on a randomized block with clones as replications.

Treatment with IAA did not significantly increase the number of propagules obtained, although the greatest number of propagules came from plants that were sprayed with IAA under continuous light and then watered with TIBA. However, TIBA treatment significantly increased the number of propagules obtainable with either pre-treatment.

The mean differences between clones, over all treatments, showed that clones 9 and 13 (late-flowering growth types) produced significantly more propagules than clones 12 and 23 (early-flowering growth types).

(ii) *Treatment of plants with TIBA and MH under field conditions*

In the following experiments, propagules were taken from field-grown plants of different clones in October, 1954. After rooting, the propagules were stored over winter (1954-1955) in the greenhouse under natural day-length, in vermiculite in standard flats. Nutrients were added fortnightly when watering. These plants were transferred to the field in the second week in June and planted 3 feet apart within and between rows. They were given one of the following spray treatments:

TIBA 100 = sprayed with 100 p.p.m. TIBA on August 6 and August 23, 1955.

MH 300 = once-weekly spray: 100 p.p.m. MH from June 22 to July 6, 1955; 300 p.p.m. MH from July 13 to July 28, 1955.

MH 800 = once-weekly spray: 100 p.p.m. MH from June 22 to July 6; 300 p.p.m. MH from July 13 to July 28; 800 p.p.m. MH on August 6 and August 23, 1955.

Control = sprayed weekly with water.

The control and growth regulator treatments included a wetter, *Ultra-wet 60L*, and were applied to plants of the different clones with a 3-gallon capacity air-compression sprayer on the dates noted.

Experiment 1

To obtain estimates of their potentialities for vegetative propagation, as many propagules as possible were taken from treated field plants of clones 9, 339, 13, 64, 12, 23 selected at random between October 15 and 21, 1955; the results in Table 2 refer to individual plants. Three random samples of 100 propagules each were taken from the total number of propagules obtained from each plant. Clones 13, 64, 12, 23, 9, 339 were treated with TIBA 100, but only clones 9 and 339 were treated with MH.

TABLE 2. — NUMBER OF PROPAGULES OBTAINED AND PER CENT ROOTED AFTER SPRAYING FIELD PLANTS WITH WATER (CONTROL), TIBA, OR MH*

Clone	Growth type	Number of propagules per plant		Mean per cent rooted		Estimated no. rooted per plant	
		Control	TIBA 100	Control	TIBA 100	Control	TIBA 100
13	0	870	594	72.3	61.0	629	362
64	0	750	1133	56.3	76.7	422	869
12	III	583	772	28.0	72.7	163	561
23	IV	308	599	40.9	58.3	125	349
9	I	1463	1681	63.3	65.3	926	1098
339	III	772	1090	44.0	53.0	340	578
Mean		791.0	973.1	50.8	64.5	434.1	636.1
		MH 300	MH 800	MH 300	MH 800	MH 300	MH 800
9	I	1649	1878	68.0	70.3	1121	1320
339	III	828	1460	53.0	58.0	431	847
Mean		1238.5	1669.0	60.5	64.2	776	1083

*For explanation of growth regulator treatments, see text.

The analysis of variance of the per cent of propagules rooted from control and TIBA-treated plants was based on a split-plot design in which the main plots were clones and the sub-plots were treatments. There were significant differences between clones and the greater per cent rooting of propagules from TIBA-treated plants was highly significant.

There was a highly significant interaction between TIBA treatment and clones; this was explicable since the propagules obtained from TIBA treatment of clone 13 showed a lower per cent rooting than those obtained from the control. Clone 13 was very late in development in the first year of planting in the field and mainly crown growth was present. In clone 13, therefore, TIBA primarily reduced the size of propagules and the ease with which they could be isolated. The per cent rooting of control propagules of clone 13 was higher than in any other clone. In the other clones, particularly clone 12, TIBA increased the amount of crown bud formation and resulted in fewer and less woody stems at the time that propagules were taken; these stems possessed a greater amount of lateral bud and shoot growth, thus providing a greater number of propagules.

There were no significant differences between clones of growth types 0 and I, or between III and IV, in per cent of propagules rooted (with control or TIBA treatments), but the differences between clones of the two groups were significant.

Plants of clones 339 and 9 that were treated with MH 300 were relatively normal in appearance. In contrast, many inflorescences aborted on plants that were treated with MH 800, and a much greater number of lateral buds were present as compared with the controls, because MH had hastened onset of the second cycle of vegetative growth. After MH 800 treatment, leaves were reduced in size, irregular in outline, thicker, more brittle, and pale bluish-green in colour. These effects were less apparent in clone 339 than in clone 9. The length of life of MH 800-treated stems was greater, the lateral buds could be readily detached from the stems, and there was pronounced formation of root primordia at the base of some of the lateral buds. The rooting and subsequent growth of propagules from MH-treated plants were normal. Apparently, MH 800 arrested growth and differentiation of apices at a certain stage of floral differentiation, but vegetative growth continued subsequently at the less differentiated higher order apices.

Experiment 2

In this experiment, all possible propagules were taken from entire primary stems of control and MH 800-treated plants of clone 9 on October 21 and 22, 1955. Three plants from both treatments were evaluated by taking all possible propagules from two stems of each plant. Results were noted on November 9, 1955, for number of propagules obtained, the per cent rooted, and the estimated number of propagules rooted per plant (Table 3). The "estimated number rooted" was calculated from the total number of propagules rooted from one stem multiplied by nine, because the mean number of primary stems per plant was nine.

The number of propagules obtained and the per cent that rooted were greater after MH 800 treatment. The estimated number rooted per plant indicated that a level of over 1,000 rooted propagules could be obtained from clone 9 after MH treatment. This value accords with the estimated number for MH treatment shown in Table 2. The increases due to MH treatment were significant ("t" test for paired variates).

TABLE 3. — CONTROL AND MH 800-TREATED PLANTS OF CLONE 9. NUMBER OF PROPAGULES OBTAINED PER ENTIRE PRIMARY STEM, PER CENT ROOTED, AND ESTIMATED NUMBER ROOTED PER PLANT (NUMBER ROOTED PER PRIMARY STEM × 9)

Replication	No. of propagules per primary stem		Per cent rooted		Estimated no. rooted per plant	
	0	MH 800	0	MH 800	0	MH 800
Plant 1	176	241	55.2	46.9	864	963
Plant 2	112	265	52.7	63.6	522	1473
Plant 3	138	248	47.0	54.6	630	1170
Mean	141.8	251.2	51.6	55.4	672	1202

Treatment and Rooting of Propagules

(i) *Influence of growth type of parental plant and position of basal cut below node on rooting of propagules*

Twelve propagules were taken from each of 10 field plants of growth types I, II, and III, respectively, in October 1953 (total of 360 propagules). The propagules were taken from the middle and basal portions of the primary stems and lower lateral shoots to ensure as much uniformity as possible among clones and growth types. One hundred and eighty of the propagules were taken with the basal cut including a quarter-inch of internode below the stem node. The experiment was designed as a randomized plot and replicated twice.

Propagules were placed in vermiculite in propagation frames on October 9, 1953, without a mist spray; results were noted on November 9, 1953. Table 4 records the mean values of per cent of propagules rooted and number of roots per rooted propagule, according to growth type and the amount of lower internode. The significance of differences was calculated from the mean values of individual clones within each growth type over all treatments.

The per cent of propagules rooted and the mean number of roots per rooted propagule were significantly greater in growth types I and II than in III ($P < 0.01$ and $P < 0.05$ for per cent rooted and root number, respectively). The per cent of propagules rooted and the mean number of roots per rooted propagule were significantly greater when cuttings were taken with a quarter-inch of the basal internode ($P < 0.01$).

(ii) *Influence of talc, sucrose, and rooting "hormones"*

Equivalent samples of 196 stem cuttings were taken from each of 14 different clones that had been maintained under 16-hour LD in the greenhouse. Twenty-eight propagules of each clone were treated with each of the following six compounds, respectively (the control was untreated propagules): talc; talc with sucrose (20 per cent by weight); "Rootone"; "Rootone" with sucrose (20 per cent by weight); "Auxan"; "Auxan" with sucrose (20 per cent by weight). "Rootone" contains NAA. "Auxan" contains IAA. The basal ends of the propagules were dipped in the

TABLE 4. — PER CENT OF PROPAGULES ROOTED, AND MEAN NUMBER OF ROOTS PER ROOTED PROPAGULE, FROM GROWTH TYPES III, II AND I. BASAL CUT AT NODE OR QUARTER-INCH BELOW.

Growth type	Per cent rooted			Number of roots per rooted propagule		
	0″	¼″	Mean	0″	¼″	Mean
III	30.0	50.0	40.0	2.8	4.8	3.8
II	76.6	76.6	76.6	5.1	6.6	5.9
I	70.0	76.6	73.3	3.5	7.0	5.3
Mean	58.9	67.7	63.3	3.8	6.1	5.0

TABLE 5. — STEM CUTTINGS PRETREATED WITH DIFFERENT "ROOTING" COMPOUNDS. PER CENT OF PROPAGULES ROOTED AND MEAN NUMBER AND LENGTH OF ROOTS PER ROOTED PROPAGULE.

Treatment	Per cent rooted	Mean no. of roots	Mean length of roots
"Auxan"	76	8.3	5.4
Talc	70	7.2	5.4
Talc with sucrose	69	6.8	5.3
"Auxan" with sucrose	67	8.7	5.3
"Rootone" with sucrose	67	7.5	4.2
Control	56	6.9	4.9
"Rootone"	55	6.2	4.0

powder, the excess shaken off, and then the propagules were transferred to vermiculite. The rooting period was from March 7 to April 7, 1953.

Table 5 records the mean values of per cent of propagules rooted and the mean number and length of roots per rooted propagule. The per cent of propagules rooted was in the order: "Auxan" > talc > talc with sucrose > "Auxan" with sucrose, and "Rootone" with sucrose > control > "Rootone". "Rootone" with sucrose increased the per cent of propagules rooted, and the mean number of roots, above that of either "Rootone" or control treatments. The general trend in the mean number and length of roots followed that of the per cent of propagules rooted, with the exception that "Auxan" with sucrose resulted in the greatest mean number of roots.

Maintenance and Storage of Rooted Propagules

The success of any large-scale method of vegetative propagation will depend partly on the efficiency with which rooted propagules can be maintained — until such a time as they are required for use.

In the autumn of 1954, about 8,000 propagules were taken from field-grown clones of different growth types. These propagules were rooted and maintained in vermiculite in standard flats, about 240 per flat for the larger propagules and 420 for the smaller ones. All flats were watered once every fortnight with nutrient solution, but otherwise with water. Propagules were either maintained under natural daylight in the greenhouse (*ca.* 13-20°C., alternating night-day temperature) or were transferred to a cold-storage cellar under fluorescent light of *ca.* 150 foot-candles, with a natural daylength regime maintained by a timed-switch. The temperature in the cold storage from November to March was *ca.* 1-5°C., rising to 5-10°C. in April and between 10-18°C. in May, 1955.

Counts taken in May-June, 1955, showed that at least 60 per cent of the propagules survived under greenhouse conditions, and at least 40 per cent in the cold storage.

A proportion of the propagules from both treatments were transplanted to the field in May-June, 1955. They produced plants that were very satisfactory for further vegetative propagation and for seed production.

There were no differences in the final growth of field plants that could be attributed to differences in their storage over winter.

Those propagules not utilized in field plantings were maintained in the original flats in open frames throughout the summer of 1955. A semi-dormant stage of growth continued to be evident and only the propagules of early growth types produced any flowering stems; in general, survival was good.

DISCUSSION

Overhead mist-spray systems for propagation work (7, 8, 13) enable very small units such as "leaf-bud" propagules of red clover to be rooted, leading to economy in space and material, as well as greater uniformity of clones. Rooting can take place in full sunlight, thus favouring rapid growth. Without the mist-spray system, shading and restriction of ventilation may be necessary to reduce wilting.

The authors are not aware of any previous reference to the use of leaf-bud propagules for vegetative multiplication of leguminous forage crops. The developmental stage of parental plants from which propagules are taken is important; if flowers are initiated, no vegetative primordia may be present (4). This explains some of the variable results of other workers [1, 9, 11, 15, and Vasters *in* Scholz and Smidrkal (12)]. Travin (15) observed that cuttings taken in spring tended to flower frequently and set seed, with only poor rosette formation, but cuttings taken from rosettes of plants in the autumn and preserved over winter, gave more satisfactory results. Scerbakova (11) found that stem cuttings did not form rosettes but that cuttings from the rosette showed the best rooting and tolerance of environmental conditions. These results can be explained on the basis that, with stem cuttings taken in the spring, there will be a continuation of stem elongation and flowering, particularly under the long daylengths normally operative under field conditions. When large buds or portions of stems of red clover are taken as propagules, root formation may be restricted to the lower cut surface of an internode, while all buds may occur on the aerial portions of the stem. Buds on the stem will produce roots only if they are sufficiently submerged in the rooting medium or soil. Potentially unlimited crown bud formation can only arise from rooted non-flowering buds. In contrast, crown cuttings taken in the autumn and stored over winter will have produced crown buds during the short days of winter, and will produce further crown growth under field conditions before flowering.

Ideally, leaf-bud propagules should be obtained from the second cycle of vegetative growth on a stem. At this stage, indeterminate growth occurs (4), many of the axillary buds are extremely small, and the leaf with its stipules and axillary bud can be readily detached from the stem. Leaf-bud propagules may also be obtained from crown buds of the first cycle of growth provided that floral initiation has not occurred.

As previously shown, the highest percentage rooting of propagules may be obtained by pre-treating parental plants with 8-hour daylengths and

then placing the propagules under 16-hour daylengths (5). Root formation may be increased by pre-soaking propagules in IAA (5) or by treating them with a proprietary "rooting hormone" (Table 5).

The present results have conclusively shown that pre-treatment of intact plants with growth regulators can very significantly increase the number of rooted propagules subsequently obtained, and that very satisfactory rooting can be obtained without direct treatment of propagules with auxin. Barrales and Ludwig (1) referred to the possible inconvenience of artificially shortening the daylength as a method of increasing crown growth – before taking propagules. Pre-treatment of plants with growth regulators obviates this requirement. Treatments with TIBA at concentrations between 25 and 100 p.p.m., either by watering the soil of plants in the greenhouse, or spraying plants in the field, significantly increased the number of propagules obtained (Tables 1 and 2). Under greenhouse conditions during the winter, the percentage rooting from TIBA-treated and control plants was similar. Under field conditions, the per cent rooting from TIBA-sprayed plants was significantly higher, due to the retardation in development, which increased the proportion of propagules consisting of bud rather than stem growth.

When plants were treated with MH under field conditions, onset of the second cycle of vegetative growth on stems was hastened due to abortion of floral apices. This increased the proportion of buds to nodes so that the number of propagules obtained and rooted was significantly greater. The most striking increases were caused by 800 p.p.m. MH applied at a time when stem elongation and flowering had begun.

Although both TIBA and MH can extend the period during which desirable propagules may be obtained, it should be noted that TIBA retards, while MH hastens onset of the second cycle of vegetative growth. Wittwer (19) found that "bolting" sugar beets sprayed with 1000-5000 p.p.m. MH did not show any further floral development and there was "reversion" to an apparently vegetative stage.

As an example of potentiality for vegetative propagation in red clover, it was found in two successive years (1954, 1955) that over 1000 rooted propagules could be obtained from field-grown plants of clone 9 (growth type I), if the whole plant was used at one time (Tables 2 and 3).

In general, the number of propagules obtained and their percentage rooting was higher in the late- as compared with early-flowering growth types. In very late-flowering types lacking stem elongation, the efficacy of MH would probably be less because the desirable effect is obtained when plants reach the flowering stage (4).

In conclusion, it is suggested that the methods and techniques outlined in these studies may be applicable to other leguminous crop plants.

ACKNOWLEDGEMENTS

The authors wish to thank those who extended help during the course of these investigations; in particular, the Goldsmiths' Company of Great Britain for the award to the senior author of a Travelling Scholarship for

the years 1952-1954; the Province of Quebec Agricultural Research Council for providing a research grant; L. C. Raymond, formerly Chairman of the Department of Agronomy, Macdonald College, McGill University, for the facilities granted and for his helpful advice; D. J. C. Friend, Plant Research Institute, Ottawa, for his helpful suggestions in reviewing this manuscript.

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A NEW CONTINUOUS POLLEN SAMPLER[1]

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[Received for publication July 10, 1961]

ABSTRACT

A new continuous volumetric pollen sampler is described which eliminates considerable labour when studying diurnal variations for long periods. Preliminary results using this new apparatus at Ottawa in late August and September showed definite hour-to-hour *Ambrosia* pollen variations with peaks up to nearly 400 pollen per cubic yard of air occurring in the early morning.

INTRODUCTION

Considerable information has been gathered in the last few years (2, 3) on the present distribution of ragweeds, *Ambrosia* spp., in Canada by field and air-borne pollen surveys across the country. The standard gravity type of collecting apparatus (Durham apparatus) as recommended by the pollen survey committee of the American Academy of Allergy (1) has been widely used in these surveys of air-borne pollen. With this apparatus slides are exposed for 24-hour periods.

To obtain further information from any given locality over a long period at more frequent intervals on the distribution and abundance of air-borne pollen and to investigate correlations between environmental factors and pollen incidence a more efficient type of collecting apparatus is required. In this paper a new continuous volumetric pollen sampler designed to meet these requirements is described; preliminary results with *Ambrosia* pollen using this new sampler are also reported.

Several types of pollen-collecting apparatus have been described in the literature and their evaluation and efficiencies have been discussed (5, 9). However, there is little published information on the abundance and types of air-borne pollen encountered using one of the more highly efficient samplers such as the rotobar. In order to conduct hourly pollen dispersal studies with the rotobar apparatus over long periods of time, considerable manual labour would be required.

Smith and Rooks (11) were successful in studying hourly variations of ragweed pollen at Iowa City, Iowa, during August and September, 1952, using a continuous recording volumetric particle sampler previously described by Stenburg and Hall (12). Hyde (7) was able to obtain much more air-borne pollen data in South Wales with the Hirst automatic volumetric spore trap (6) than with the standard gravity type of apparatus. By combining desirable features from several of these machines, including one recently described by Pady (10), and incorporating some modifications, the senior author has designed a new continuous volumetric pollen sampler. This apparatus, although made of inexpensive materials, required considerable detailed work and construction time.

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[1]Contribution No. 15 from the Engineering Research Service, Contribution No. 156 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.
[2]Engineering Research Service.
[3]Plant Research Institute.

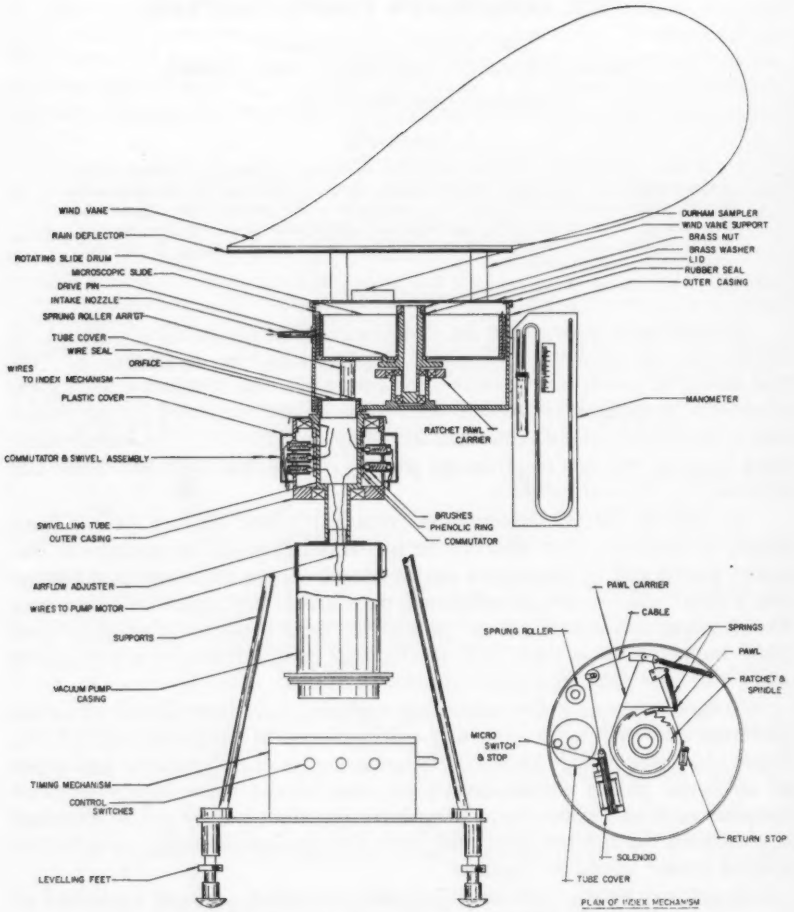

FIGURE 1. Sectioned elevation, continuous pollen sampler.

One of the chief advantages of this new sampler is that a drum can be loaded with 24 slides in the laboratory and then can be interchanged without loss of time or danger of contamination with the drum containing the exposed slides in the machine. Each slide is in a separate slot and is exposed for a definite period (15 minutes to 24 hours). This is a considerable advantage over other designs where the pollen is deposited on a single, slowly moving slide from which it is difficult to determine precisely how much has been trapped during a particular period of time.

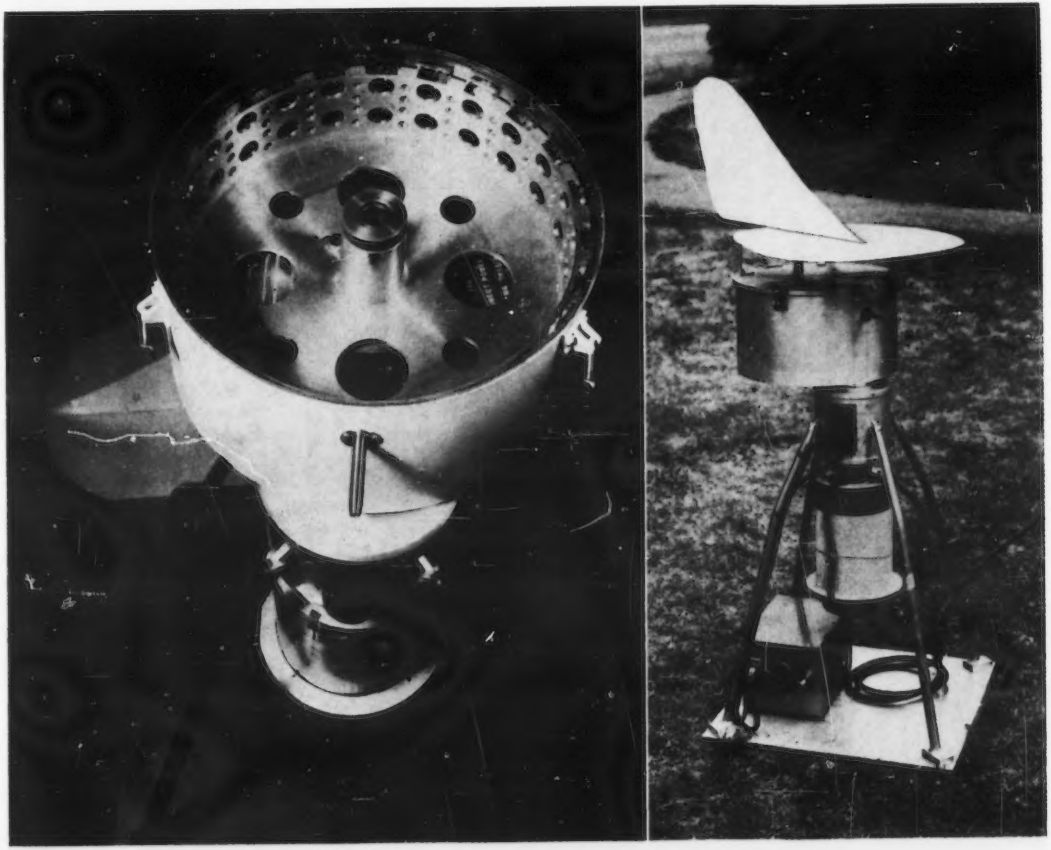

FIGURE 2. Continuous pollen sampler.

Description of Apparatus

The complete sampler is shown in Figures 1 and 2. The apparatus is self-contained, portable and will operate in any location where electric power is available. Utilizing standard 1 inch x 3 inch microscope slides, coated with a thin layer of glycerine jelly to trap pollen admitted by a 0.25-inch diameter nozzle 1 metre above the ground, the sampler operates as follows. (Figure 1 is a sectioned elevation). Slots around the periphery of an aluminum drum accommodate 24 slides. The drum is driven by a ratchet wheel integral with its spindle and rotates each time the indexing mechanism operates. The simple indexing mechanism is driven by a solenoid, while a sprung roller drops into a detent at each slide position, preventing continuous rotation and aligning the slide with the nozzle axis. Slide drum and indexing mechanism are mounted in an air-tight chamber attached to the top of a swivelling tube. A wind vane, rain shield and a standard gravity type slide holder are mounted on the lid assembly. The complete assembly is thus able to turn the nozzle into the prevailing wind. Air is drawn from the air-tight chamber by a vacuum cleaner motor and blower establishing flows up to 26 l.p.m. through the nozzle whose face is 1 millimetre from the slide. Flow is controlled by bypassing air to the pump and indicated by a calibrated manometer.

Calibration and Design

The nozzle length was arranged so that maximum streamline deviation at its entry was 5° when considering flow around the outer case. This compromise was considered satisfactory, since pollen was not found to stick inside the polished stainless steel nozzle and the slides were coated fairly evenly over a 0.25-inch diameter area.

The exposure of one slide for a period of 8 hours in front of the nozzle did not reveal any trace of dust or pollen on adjacent slides inside the drum. The movement of one slide to the next slot also did not result in any contamination on the unexposed slides inside the drum.

The efficiency of the apparatus was compared with that of a cascade impactor (8) using a similar technique to that of Gregory (4), who determined the trapping efficiency of cylinders. Insufficient data were obtained to give accurate efficiencies, since the large wind tunnel used was not suitable for the purpose. Sampling at 6.8 l.p.m. gave the best results at wind speeds between 4 and 12 m.p.h., i.e. the sampler operated isokinetically at 8 m.p.h.

Pollen Results

The new sampler was located at ground level at the agrometeorological station, Central Experimental Farm, Ottawa. In Figure 3, hour-to-hour variations in the amounts of *Ambrosia* pollen trapped are shown for August 23-24 and September 22-23, 1960. Similar results were obtained on August 29-30, as shown for August 23-24. The low pollen peaks on September 22-23 are probably due to the fact that the ragweeds had already passed their maximum flowering period. Weather conditions for each day

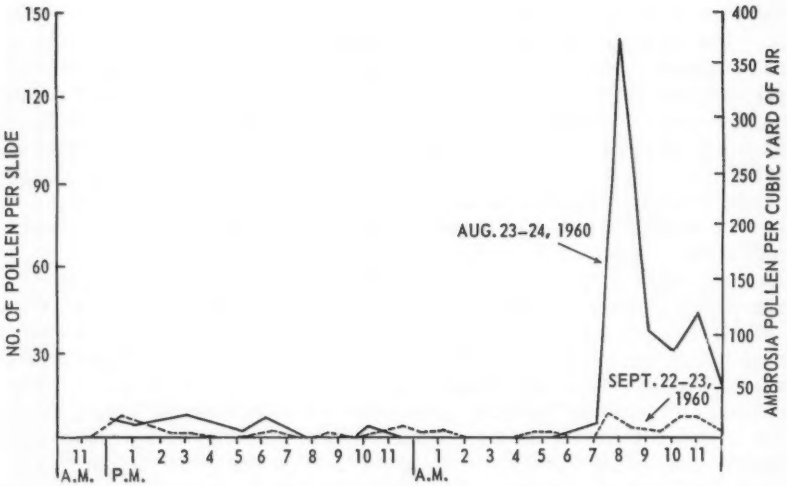

FIGURE 3. The number of *Ambrosia* pollen per hour collected on August 23-24 and September 22-23, 1960.

of operation were about normal for this area with temperatures above 68°F., relative humidity over 65 per cent, light winds, sunny and cloudy periods, with no rainfall.

The peak pollen periods, in all cases, were in the early morning between 7 and 9 o'clock. After these peak periods there was a tapering-off during the rest of the day with very little *Ambrosia* pollen collected at night-time. Further studies are being continued to determine whether or not these preliminary results are typical. The number of pollen grains per cubic yard of air was calculated from the number of pollen per slide with the machine operating at a constant air-intake of 4.8 l.p.m.

Although the percentage efficiency of this new sampler was not obtained accurately, it is felt, from tests conducted, that its efficiency is similar to that of the Stenburg and Hall apparatus which was reported to be over 70 per cent.

ACKNOWLEDGEMENTS

The authors wish to thank D. C. MacPhail, Director, Division of Mechanical Engineering, National Research Council, for permission to use one of their wind tunnels for testing the new sampler and for the services rendered by his staff.

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ROOT AND STALK ROT OF FIELD CORN IN SOUTHWESTERN ONTARIO

II. DEVELOPMENT OF THE DISEASE AND ISOLATION OF ORGANISMS[1]

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[Received for publication May 29, 1961]

ABSTRACT

The development of root and stalk rot in two corn inbreds was followed in 1956 and isolations were made of organisms associated with the disease. Infection of the root tips early in the life of the plants was followed by progressive colonization of the roots as the plants aged and by rotting of the stalks at maturity. Isolations showed that numerous organisms (*Fusarium oxysporum*, *F. moniliforme*, *F. equesiti*, *F.* sp., *Pyrenochaeta terrestris*, *Trichoderma* spp. and *Alternaria* sp.) were associated with the rotting of the roots but that only *F. oxysporum* and *Trichoderma* spp. were obtainable from the stalks of diseased plants. The two latter organisms entered the stalks through diseased adventitious roots or mesocotyls and were considered to be the cause of the disease in 1956. The association of other organisms in this complex disease in other years is discussed.

INTRODUCTION

In 1953, McKeen (4), reporting on studies on root and basal stalk rot of maturing corn carried out at Harrow, Ontario, stated that *Pythium arrhenomanes* caused a necrosis of the roots which later advanced into the stalks. In 1957, Whitney and Mortimore (8) reported, also from Harrow, that root and stalk rot are successive phases of a disease of corn which begins in the roots and later spreads into the stalk as the plant matures. They, however, had consistently isolated two species of Fusarium as well as one of Pythium from the roots and stalks of diseased plants. The uncertainty as to the organisms associated with the disease in an apparently causal relationship is accentuated by the findings of certain workers elsewhere. For example, Ho and Melhus (1) in 1940 in Iowa, in an investigation on the succession of soil-inhabiting fungi attacking the roots of maize, found that numerous species were involved and that "on host maturity *Diplodia zeae*, *Fusarium* spp., etc., may develop extensively in roots and crown, leaving no evidence of earlier prevalent organisms." Also, in 1952, in Iowa, Summers (7) reported that the fungi isolated most frequently in August and September from the root tips and the stele of secondary roots and from the lower three internodes of the stalks of diseased plants were *Pythium graminicola*, *P. arrhenomanes*, *Gibberella saubinettii*, and *Fusarium* spp.

The present authors have confirmed that natural infection of the roots occurs early in the life of the corn plant and that infection or colonization of the root system continues throughout the remainder of the growth period. The organisms associated with these phenomena of root pathogenesis and the relationship between such organisms and those that can be isolated from the stalk are not well known. The present paper reports what was observed critically on the development of root and stalk rot and what organisms were found to be associated with the disease in 1956.

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MATERIALS AND METHODS

Roots and stalks of two dent inbreds, M13 and WF9, susceptible to root and stalk rot were sampled for disease ratings and isolation of fungi and bacteria fortnightly from July 3 to October 9, and for a final disease rating on October 30. A split-plot, four-replicate, experimental design with sampling dates as sub-plots was used. Each plot consisted of an 8-foot row in which individual plants were 1 foot apart. The seminal and adventitious roots of five plants of each inbred in each replicate were examined for disease on each date. A rating system was used to assess the disease on the adventitious roots, independent ratings being made for the crown and brace roots. At each sampling date stalks of four plants of each inbred in each replicate were cut longitudinally and examined for disease. Root isolations were made on July 3 from the seminal roots and adventitious crown roots, on July 16, 30, and August 13 from the adventitious crown roots and their branch roots, and on the remaining dates from the adventitious crown roots, adventitious brace roots and branch roots from both. In each case isolations were made from six diseased segments. Stalk isolations of bacteria and fungi were made from the interior of the crown, and from the first and second elongated internodes on each examination date, except August 27, until typical stalk rot symptoms appeared. On September 10, M13 plants showing typical stalk rot were selected to determine the fungi actually associated with the final syndrome in the diseased plant.

Preparatory to attempted isolations selected sections of roots and stalks were brushed clean with soap and water, rinsed once in tap-water and once in sterilized water, immersed in mercuric chloride solution (1:1000) for 1 minute and rinsed twice in sterilized distilled water.

Isolations of all fungi, except Pythium, were made on potato dextrose agar (PDA). Since this medium did not suppress growth of bacteria which could not be eliminated from the decayed interior of the surface-sterilized root, a special technique was developed for the further handling of some of the specimens treated as described above. Individual roots and rootlets, or segments of each, showing evidence of the disease were dipped in melted paraffin (55 C), immersed in cool water (18 C), and stored in sterilized receptacles. The complete operation was carried out under asceptic conditions. Lesions on roots so treated were clearly visible through the wax, thus facilitating ready choice of suitable material. In making isolations a waxed root was severed through a lesion with a flamed razor blade and the disjoined segment was transferred to a screw-cap test tube containing an agar slant. The lesioned end of the segment was positioned 1 millimetre from the edge of the agar slant. The tube was flamed lightly in the region of the segment; the wax melted momentarily but then, hardening, held the segment firmly in position. After 2 or 3 days fungus grew from the exposed end of the segment into the agar. Mycelium from the advancing edge of the colony was then transferred to another agar slant. In nearly every case a fungous culture free from bacteria was obtained.

For the isolation of Pythium free from bacteria, the following technique was used. Sections of roots previously washed and surface-sterilized were

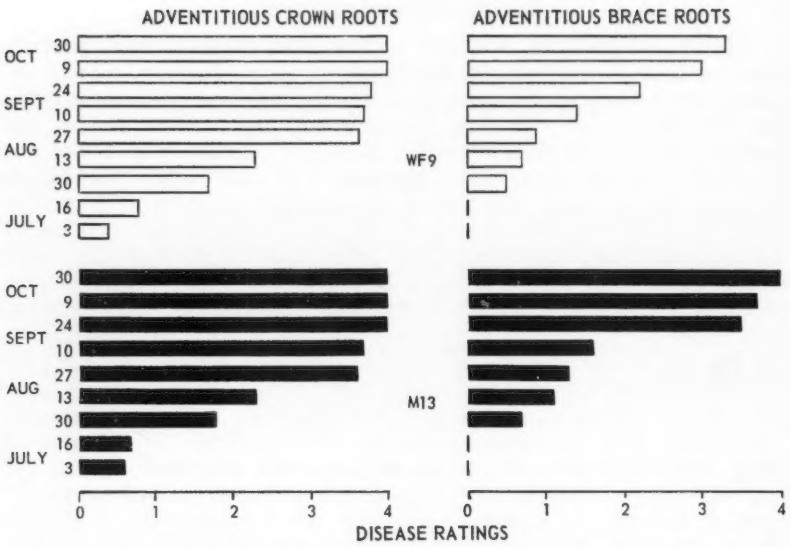

FIGURE 1. Disease ratings of the adventitious crown and brace roots of two corn inbreds, 1956. 0 — no disease; 1 — disease restricted to the root tips; 2 — disease restricted to the lower half of the roots; 3 — some of the roots totally diseased; 4 — all of the roots totally diseased.

immersed in gently flowing tap-water overnight. Selected segments were transferred to cornmeal agar in a petri plate, each segment being then covered with a 1-inch square of the same medium. If, after incubation at room temperature, a hyphal tip had grown through and emerged from, the covering agar, it was removed and transferred to another plate of the medium.

For the isolation of bacteria, segments of washed and sterilized roots were transferred directly to PDA.

All culture work was done at room temperature.

RESULTS

Development of the Disease:

Roots

As mentioned above, roots of the two inbreds, M13 and WF9, were rated for disease at 2-week intervals, starting July 3. By July 16, the seminal roots of both inbreds had become completely diseased. The disease ratings of the adventitious roots are presented in Figure 1. In both inbreds infection occurred on the tips of the adventitious crown roots early in July. As the plants aged, the infection of these roots spread at approximately the same rate on both inbreds until all became totally diseased. Infection

occurred on the tips of the adventitious brace roots of both inbreds late in July, but in these roots the infection advanced more rapidly in M13 than in WF9.

Microscopic examination of transverse sections of the adventitious roots revealed that infection was confined to the cortex on the first two examination dates, but then the stele also became infected.

Stalks

Examination of longitudinal sections revealed that infection spreads from diseased adventitious roots or mesocotyls into the stalks (Figures 2 and 3) as the plants approach maturity. No evidence of stalk infection was noted until August 27, and then only as small, darkened areas in tissue adjacent to the epidermis in stalks of three M13 plants and one WF9 plant. Although approximately 70 per cent of the M13 plants had stalk rot by September 24, it was approximately 4 weeks later before the same percentage of WF9 plants had the disease. This supports previous findings (8) that "corn of early maturity becomes diseased before corn of later maturity", because WF9 matures about 3 weeks later than M13.

The above observations indicate certain main developmental phases of the disease, namely, infection of the root tips early in the life of the plant, progressive colonization of the roots as the plant ages, spread of infection from roots to stalks, and rotting of the stalks at maturity.

Isolation of Organisms:

Roots

From the outset of this investigation it was realized that more than one group of micro-organisms, i.e., fungi, bacteria, and nematodes, might be involved in this complex disease. However, the root isolations were restricted to fungi since perusal of the literature showed that they were the predominant organisms in root rot of corn.

Data on fungi frequently isolated in this investigation are presented in Table 1. It will be noted from this table that (a) only two species of fungi, *Pyrenochaeta terrestris* and *Fusarium oxysporum*, were isolated consistently throughout the season, whereas others were isolated either sporadically or at certain periods only; and (b) species of Fusarium were predominant.

In addition to the fungi recorded in Table 1, the following were isolated occasionally but were not thought to be of significance in the disease complex: *F. arthrosporiodes*, *Pythium* sp., *Aspergillus* sp. and *Penicillium* sp.

Stalks

Bacteria were isolated from the stalks of both inbreds on all the dates indicated. Although they were not identified, two distinct types were noted: orange-coloured colonies from the crown, and white-coloured colonies from the first and second elongated internodes.

A few fungous isolates were obtained from the stalks of M13 on July 30 and from both M13 and WF9 on August 13. They were different from those isolated from the roots and no attempt was made to identify them.

Two M13 plants showing early typical symptoms, as shown in Figures 2 and 3, provided material for a detailed study of the final syndrome of the stalk phase of the disease. In one of these plants (Figure 2), crown tissues had just become infected, either through the mesocotyl or roots in the first two whorls of adventitious crown roots. A lesioned area extending from the mesocotyl into the stalk was cut longitudinally into three strips. Isolations were made from the mesocotyl and stalk areas of each strip. *Fusarium oxysporum* was the only fungus obtained from both stalk and mesocotyl tissue. The other plant showed evidence not only of a more advanced stage of the disease but also that infection had spread into the stalk from a diseased root in the fifth whorl of adventitious roots. Outwardly this particular root was definitely rotted except for the final inch. Internally, however, the entire stele was discolored and necrotic as was also the tissues of the stalk at and beyond the juncture of the two organs. *F. oxysporum* was readily isolated from the stele of the root in question. In this plant the first elongated internode, i.e., the one between the fifth and sixth whorls of adventitious roots, was rotted and discolored, the tissue in the second was watersoaked only, while that in the third appeared normal. Isolations were made from the lower, middle, and upper areas of these internodes. *F. oxysporum* was the only fungus isolated from all excisions. *Trichoderma* spp. were isolated from a few of the excisions from all three areas of the first two elongated internodes but only from the lower area of the third internode.

The facts that *Fusarium oxysporum* was the only fungus isolated from the stalk in the early stages of stalk rot and that this fungus had advanced farther up the stalk than the *Trichoderma* spp. in the plant with more advanced stalk rot suggest that, in these cases in 1956, *F. oxysporum* might have been the primary pathogen and the *Trichoderma* spp. secondary invaders. Any role which the bacteria and fungi that were isolated from the stalks, before stalk rot symptoms were evident, might have in this disease is not known.

The results of the above isolations show that *Fusarium oxysporum* and *Trichoderma* spp. were the only fungi common to both the roots and stalks of diseased plants. This suggests that these two organisms might have been responsible for the root and stalk rot in 1956. The role that the other fungi isolated from the roots have in this disease complex is not understood.

DISCUSSION

The early and progressive colonization of corn roots by fungi (Figure 1) probably occurs consistently on field corn in southwestern Ontario since it is almost impossible to find a fully developed corn plant that does not have extensive root rot. Such a general condition of root rot in this crop might be a factor which prevents a higher yield than that normally obtained. This hypothesis has not been tested since it has not been possible to grow corn plants free from root rot in outdoor plots. The early rotting of the seminal roots might also be important in this respect because of the possible adverse effect on nutrition in the early stages of

FIGURES 2 AND 3. Median longitudinal sections of diseased roots and the lower part of the stalks of plants of inbred M13, September 10, 1956.

FIGURE 2. Note lesions extending from the mesocotyl and the adventitious roots into the crown.

FIGURE 3. Note the diseased stele of the large adventitious root on the right side and the diseased stalk tissue.

TABLE 1. — FUNGI ISOLATED MOST FREQUENTLY FROM ROOTS OF TWO CORN INBREDS ON EIGHT DATES, 1956

Date	Roots[1]	*Pyrenochaeta terrestris*		*Fusarium oxysporum*		*Fusarium moniliforme*		*Fusarium equesiti*		*Fusarium* sp.		*Trichoderma* sp.		*Alternaria* sp.	
		M13	WF9	M13	WF9	M13	WF9	M13	WF9	M13	WF9	M13	WF9	M13	WF9
July 3	S	+[2]	+	+							+				
	AC	+	+	+	+			+			+				
July 16	Br		+	+	+										
	AC		+	+	+								+		
July 30	Br	+	+			+								+	
	AC			+	+							+		+	+
Aug. 13	Br	+													
	AC			+	+	+						+	+		
Aug. 27	Br	+	+	+		+	+								+
	AC		+	+	+	+	+	+			+			+	
	AB			+				+	+		+			+	+
Sept. 10	Br	+	+		+	+	+		+					+	
	AC	+			+	+	+		+	+	+			+	
	AB			+	+	+	+						+		
Sept. 24	Br	+	+	+		+	+			+					+
	AC	+	+	+	+	+	+			+	+	+			
	AB	+	+	+	+					+			+		
Oct. 9	Br	+	+	+	+		+			+	+				
	AC	+	+	+	+	+					+	+			
	AB			+	+					+					

[1]S = seminal roots
AC = adventitious crown roots
AB = adventitious brace roots
Br = branch roots on adventitious crown roots and adventitious brace roots
[2]Each plus mark indicates the presence of the fungus.

plant growth. Mosolov and Panova (5) showed that growth of corn was retarded when the primary roots were isolated from nutrients, and Kiesselbach (2) reported that severing the seminal root system at the age of 3 weeks reduced yield of grain by 9 per cent.

The fact that the incidence of root rot on the adventitious crown roots was similar for both inbreds on each examination date and yet stalk rot occurred later in WF9 than in M13 supports the conclusion made in 1957, by the present authors, that the host must be in a late stage of its development before the disease can occur in the stalk. This conclusion is further substantiated by recent evidence* which demonstrates that stalk rot does not occur until the plant is physiologically mature as determined by maximum dry weight of the kernels.

As was implied in the introduction of this paper the types of organisms isolated from diseased roots and stalks of corn vary from year to year. In the present investigation several species of Fusarium, especially *F. oxysporum* and *F. moniliforme*, and *Pyrenochaeta terrestris* were isolated consistently from the roots and stalks. Pythium was isolated only once from the roots and *Diplodia zeae* and *Gibberella zeae*, both corn pathogens, were never isolated from the roots or stalks. However, in 1954 Pythium was present in diseased roots of corn grown in the same location (8). In 1949 and 1950 *Pythium arrhenomanes* was reported as the cause of root rot of corn in Ontario and it was also reported to have spread into the stalks (4). On the other hand *Gibberella zeae* was consistently isolated from stalks of diseased plants by the authors in 1957. Koehler (3) claimed that "in some years Gibberella is the primary cause of stalk rot and in others it is Diplodia". Because such inconsistencies occur from year to year, it is difficult to assess the role that any one, or all, of these organisms might have in this disease. It would appear that root and stalk rot can be caused by various fungi and that those fungi which are found associated with the disease in any one year might be dependent to a large degree upon certain selective environmental conditions. Accordingly, the present authors believe that in this disease complex generalization should not be made as to which organism(s) is the primary cause of root and stalk rot to the exclusion of all others, but rather that the disease should be regarded as resulting from the activity of numerous organisms which cannot be predicted with accuracy from season to season.

ACKNOWLEDGEMENT

The authors wish to express appreciation to W. L. Gordon, Rust Research Institute, Winnipeg, Manitoba, for assistance in identification of the *Fusarium* species.

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NOTE ON AN INCREASING-RATE FERTILIZER SPREADER

The establishment of plant-growth fertilizer-response curves depends upon the judicious selection of fertilizer rates. Where information concerning plant response to fertilizer under different climatic conditions and on various soils is lacking, preliminary data are often obtained to serve as a basis for the selection of the fertilizer rates. It would be a distinct advantage to omit the obtaining of preliminary data and to determine the plant response curves directly. As one development in this program a Gandy fertilizer spreader was modified to apply fertilizer at algebraically increasing rates along the length of the plot.

The spreader box (A) is mounted on a suitable carrier (Figure 1). The perforated plate (B) is moved with a ball-bearing rider (C) welded to the plate and riding on a cam (D) attached to a gear-reduction box (E) (Figures 2 and 3). The gear-reduction box and the agitators are driven through a series of sprockets and chains by a ground-driven wheel (Figure 1). The rider is held against the cam by spring tension (F) (Figure 2). The cam has a lift equal to the length of the holes through which the fertilizer drops. When the rider is against the low or high point of the cam, the holes are just closed or fully opened, respectively.

The length of ground travel required for the holes to change from fully closed to fully opened is governed by the sprocket ratio from the

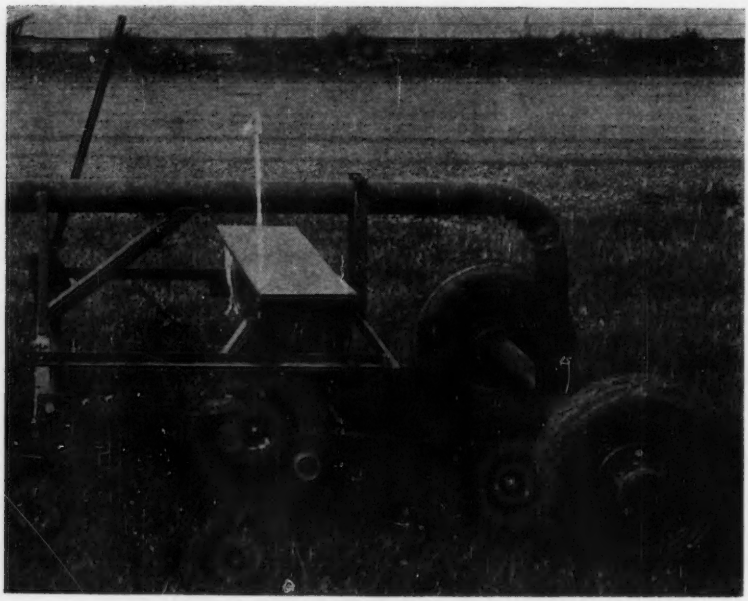

FIGURE 1. Modified Gandy fertilizer spreader box (A) mounted on a carrier.

FIGURE 2. Bottom view of spreader showing perforated plate (B), gear box (E), and tension spring (F).

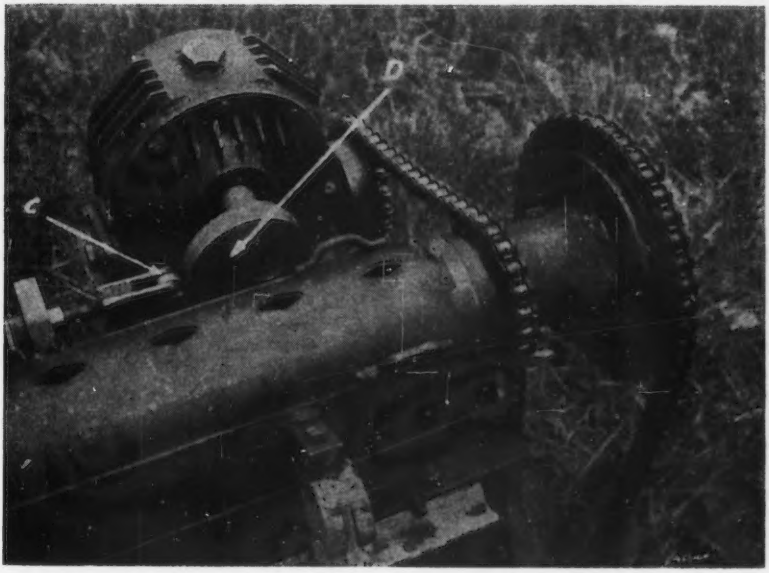

FIGURE 3. Close-up view of gear box showing rider (C), cam (D), and chain drives.

FIGURE 4. Pans distributed along length of travel and used to determine rate of application at specified distances.

ground-driven wheel to the gear-reduction box. The rate of fertilizer application in relation to length of travel is determined by weighing the fertilizer caught in standard-sized pans laid at pre-determined distances along the length of the plot (Figure 4).

The spreader has been used successfully to apply nitrogen and phosphate fertilizers to established grass plots. The use of the machine and the method of obtaining and handling yield data will be reported in detail at a later date.

ACKNOWLEDGEMENTS

Acknowledgement is made to H. Bergen and J. Kanashiro for assistance in the modification of this spreader.

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June 13, 1961

NOTE ON MALE STERILITY IN THE COMMON LOWBUSH BLUEBERRY, *VACCINIUM ANGUSTIFOLIUM* AIT.[1]

During the course of greenhouse pollination experiments on the common lowbush blueberry, *Vaccinium angustifolium* Ait., we found that 3 out of 24 plants being studied were male-sterile. This was unexpected because, to the best of our knowledge, there have been no previous reports of male sterility in the genus *Vaccinium.*

The flowers of the male-sterile plants were very similar to those of normal plants, except that the stamens were somewhat shorter, the pollen sacs were slightly shrunken, and no pollen was produced. When the pistils of the male-sterile plants were pollinated, the percentage set varied from 80.6 to 90.3 per cent, and the berries contained up to 60 viable seeds each. The female reproductive parts of the male-sterile plants therefore were considered normal.

A survey was conducted in May, 1961, to determine the frequency of male-sterile plants in commercial fields in Nova Scotia. We collected flowering shoots from individual clones in the field and brought them into the laboratory for examination. A total of 1,171 plants were examined: 5.2 per cent were male-sterile producing no pollen, 49.8 per cent examined produced abundant pollen, and the remaining 45 per cent produced amounts varying from somewhat less than normal to practically none.

Of the three male-sterile plants originally found, one was collected from New Brunswick, another from Newfoundland, and the third from Quebec. Since they also occur in Nova Scotia, one can conclude that the male-sterile factor is widely distributed in Eastern Canada. It would also appear that either the male-sterile factor is not simply inherited, or that more than one type of male sterility is present.

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[1]Contribution No. 1080 from the Research Station, Research Branch, Canada Department of Agriculture, Kentville, N.S.

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August 8, 1961

NOTE ON THE EFFECT OF STAGE OF DEVELOPMENT AND VARIETY ON DAMAGE TO BARLEY BY THE CORN LEAF APHID, *RHOPALOSIPHUM MAIDIS* FITCH

During the summer of 1955 a severe aphid outbreak occurred on barley in Western Canada. Heavy damage to late-sown barley was reported by MacNay (3) with *Rhopalosiphum maidis* Fitch, the corn leaf aphid, the principal species involved.

Barley plots at Lethbridge, Alberta, were severly infested with aphids by late July and observations were made to determine whether or not the date of seeding and the variety had any effect on the amount of damage caused by aphid feeding. Dahms *et al.* (1) have reported differences between barley varieties in reaction to damage caused by the greenbug, *Toxoptera graminum* Rondani, but no differences in reaction to corn leaf aphid have been reported for this crop.

Plots on dry land, consisting of 12 varieties of barley sown at four dates in single rows, 10 feet long and 1 foot apart, were among those severely infested by late July. The seeding dates were June 3, 6, 10, and 13, but barley sown on the last two dates did not germinate until June 24, when sufficient rain fell to moisten the surface layer of the soil. By late July, growth of varieties of the first and second dates of seeding had advanced to the late stage of stem elongation (2). Most were in the boot stage, although elongation of the upper stem internode had not occurred in some of the late varieties sown on the second date. All varieties sown on the last two dates were still in the tillering stage. There were no apparent differences in aphid infestation between varieties. Population counts were not made, but all varieties sown on all dates were rated as being heavily infested. However, a difference was noted in the distribution of aphids on barley plants at different stages of development. Aphids on plants in the tillering stage were uniformly distributed over the entire plant, whereas aphids on plants in the late stages of stem elongation were clustered on the lower portions of the leaf blades and almost absent on the stems and older tissues.

On July 27, four days after the infestation was first observed, symptoms of heavy aphid feeding, such as slight desiccation, curling, and chlorosis of the leaves, were apparent. On that date, one-half of each row was sprayed with malathion, the other half being left as an unsprayed check. Malathion was applied as a water emulsion by means of a knapsack sprayer at the rate of 1 pound of toxicant per acre. Satisfactory aphid control was obtained on the treated half-rows and no obvious reinfestation from the untreated barley occurred until approximately 10 days after spraying.

On August 17 the percentage aphid damage, as indicated by reduction in the plant growth (and consequently yield), was estimated for each plot by comparing sprayed and unsprayed plants. A rating of 0 was given where there was no difference between the sprayed and unsprayed plants. A rating of 100 was given when the plants in the unsprayed portion of the row did not develop beyond the tillering stage while those in the sprayed portion of the same row headed and matured normally.

TABLE 1. — ESTIMATED PERCENTAGE REDUCTION IN YIELD OF 12 BARLEY VARIETIES INFESTED IN THE TILLERING STAGE WITH THE CORN LEAF APHID, LETHBRIDGE, ALBERTA, 1955

Variety	C.A.N.[1]	Date of seeding	
		June 10	June 13
H 47103	—	10	0
Compana	1154	5	10
Freja	252	5	15
H 4771	213	10	10
Vantage	1162	15	20
O.A.C. 21	1086	20	20
Husky	149	30	40
Montcalm	1135	60	50
Proctor	257	25	90
Hannchen	1109	80	85
Stephan	1142	95	100
Maja	—	100	100

[1]Canadian accession number

There were no observable differences between the sprayed and unsprayed portions of the rows seeded on the first two dates where all varieties had developed to the late stages of stem elongation when the aphid infestation occurred. It was apparent that the heavy aphid infestations present had little or no deleterious effect on yield. On the other hand, in rows seeded on the last two dates, where the infestation occurred during the tillering stage, marked reductions in yield were apparent. The estimates of yield reduction caused by aphid feeding in plots seeded on the last two dates are presented in Table 1. Since barley sown on these dates emerged at the same time, the dates are essentially duplicates. The data are reasonably consistent for each variety, indicating a uniform aphid infestation. The differential reaction of these varieties to aphid feeding is also apparent.

These results indicate that little damage can be expected when aphids attack barley that has advanced to the late stages of stem elongation. On the other hand, severe damage can be expected when heavy aphid populations are present at earlier stages of development. Heavy damage to such commonly grown varieties as Montcalm and Hannchen can be anticipated if the plants are subjected to heavy aphid infestation during the tillering stage. Damage to varieties such as Compana and Vantage, which appear relatively tolerant, will probably be less severe when attacked at that stage. Spraying barley in the tillering stage with malathion to control aphids should provide protection until the crop has reached a tolerant stage of development.

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June 9, 1961

NOTE ON A CHEMICAL TREATER FOR SMALL SEED SAMPLES[1]

A mechanism was developed to treat with chemical powders small packets of seed used in cereal crop research projects. These powders are either toxic or produce objectionable dusts to which personnel may be allergic, a problem taken into account in the construction of the dispenser. In addition, they tend to cake in a container and the problem is to make them flow consistently in quantities suitable for adequate treatment.

DESCRIPTION OF APPARATUS

The dispenser shown in Figure 1 is in a fume cupboard with a transparent front. It has built-in lighting and a ½-h.p. fan for removing fumes and floating dust by exhausting them outside the building via a flexible duct. The unit is portable and can be used where air and electric supplies are available.

A section of the dispensing unit is shown in Figure 2. The seed packet is held below the dispenser nozzle (A), and a micro switch (B) is operated by depressing a plate (C) behind the nozzle. This energizes a solenoid (D), pulling the dispensing shaft (E) through the hopper (F) of chemical

[1]Contribution No. 16, Engineering Research Service and No. 68, Genetics and Plant Breeding Research Institute, Canada Department of Agriculture, Ottawa, Ont.

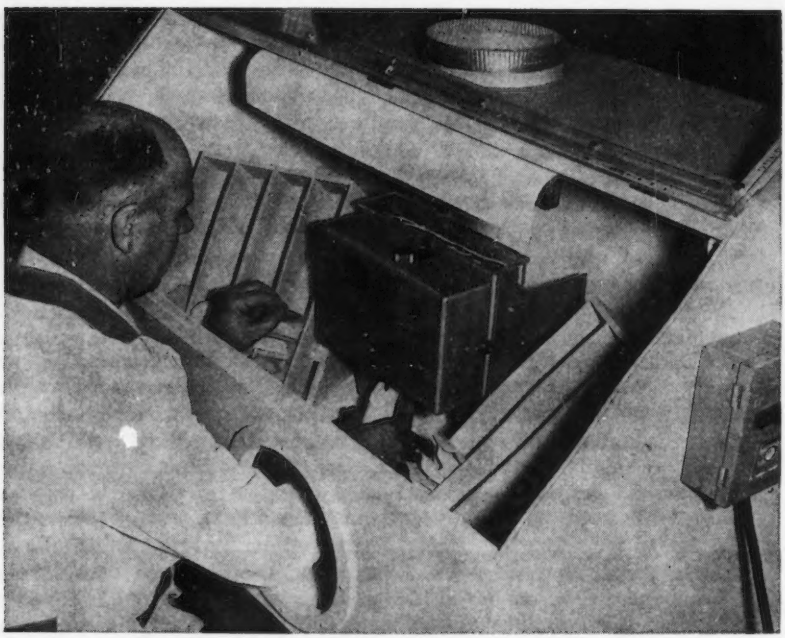

FIGURE 1. Chemical seed treater for small seed samples.

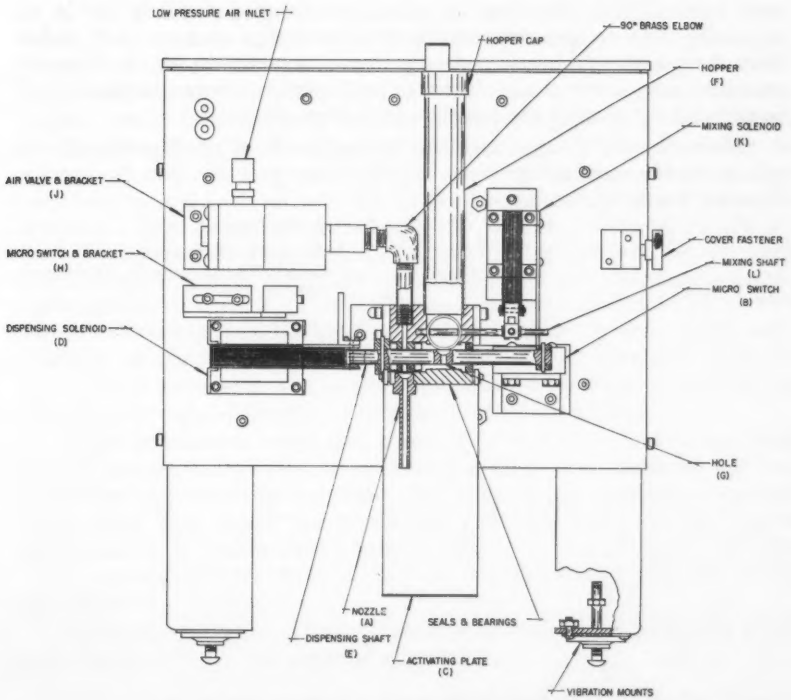

FIGURE 2. Sectional drawing of the dispensing unit.

powder which has fallen into a hole (G) in the shaft. When the hole is in line with the dispensing nozzle (A), a second micro switch (H) opens a valve which admits low pressure air. Air puffs the powder through the hole out of the nozzle into the packet. As this process is taking place, a second solenoid (K) energized by the first micro switch (B) rotates an agitator (L) at the bottom of the hopper. Releasing the plate (C) de-energizes the two solenoids (which are returned by springs) and valve; the dispenser is then ready for the next packet. A vibrator made from a 1/250-h.p. motor turning an out-of-balance fly wheel vibrates the dispenser to keep the agitated powder falling down into the dispenser shaft hole. Suitable vibration mounts isolate the dispenser from the cabinet. An electromagnetic counter operates as each packet is treated to simplify recording the number of samples.

The unit was used to dispense approximately 0.04 gram of powder. To test the consistency of operation a mercurial fungicide was puffed into 100 empty seed packets. The mean weight of powder dispensed was 0.038 gram and the C.V. was 17 per cent. On the basis of treating 10 grams of wheat, this would be at a rate of 3.5 ounces per bushel. The amount of powder dispensed is limited by the ability of the powder to drop into a

small hole. The amount can be varied by changing the hole size in the dispensing shaft or operating the dispenser more than once for each packet. Since the quantity dispensed is already close to a minimum for the dispenser, treatment rates lower than 3.5 ounces per bushel for some chemicals could be adjusted by diluting the powders with inert material.

Normally the dosage treatment of dry seeds in small packets is not critical within reasonable limits. Puffing the powder into the packets disperses it over the seed.

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July 28, 1961

NOTE ON A NEW MITE DISEASE OF BARLEY AND OTHER CEREALS[1]

A new species of spider mite, *Tetranychus* sp.[2] (Acarina: Tetranychidae) heavily infested barley fields at Winnipeg, St. Jean, Culross, Carman, Morden, Portage la Prairie, Brandon and Dencross in Manitoba during the summer of 1961. It is greenish-yellow to orange in colour with a large, dark longitudinal band on each side of the dorsum. It produces very little webbing. Eggs are spherical and opaque. In many fields, 90 to 100 per cent of the plants were attacked; it occurred in large numbers, e.g. 347 mites and 271 eggs in approximately 80 square millimetre leaf area, usually on the under surface of the leaf. Feeding causes a darkening, followed by yellowing and withering from the bend of the leaf on to its tip and, occasionally, crinkling of the leaf edges from below. Often the entire area along the two sides of the midrib and isolated patches in other parts of the leaf are affected this way. Symptoms resemble those of net blotch.

Light infestations were also observed in rye, corn and wheat fields near Winnipeg, but no infestations were found in oats. In barley and rye, symptoms appeared to be due to the toxic effect of mite feeding. In wheat, fewer mites were found but mosaic-like yellowing of the leaves suggests the possibility of a transmissible virus infection. Severe drought conditions in Manitoba may have some bearing on the widespread outbreak of the leaf disease.

Infestation patterns and mite resistance in varieties of barley are under investigation and will be reported elsewhere.

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[1]Contribution No. 91 from the Research Station, Canada Department of Agriculture, Winnipeg, Man.

[2]Baker, E. W. (in litt.), who relates it to *Tetranychus canadensis* (McGregor), has kindly consented to describe the species in a later paper.

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August 21, 1961

NOTE ON THE CREAMETTE FIELD PEA[1]

Creamette field pea was developed at the Central Experimental Farm, Ottawa, Ontario. This yellow-seeded variety of good quality possesses agronomic characteristics of early maturity and high yield. In addition, it exhibits considerable tolerance to the leaf and pod spot disease caused by the fungus *Ascochyto pisi* Lib.

BREEDING HISTORY AND PEDIGREE

The parents of Creamette, previously known as 3884 A, are Chancellor, Early Raymond and Stirling. The first cross between Chancellor and Early Raymond (Pedigree No. 1187) was made by J. G. C. Fraser in 1924. From this population one selection was made. In 1942, P. R. Cowan crossed the above selection with Stirling (Pedigree No. 3884); from the resulting bulk population selection A was established. In 1955 3884 A was included in the Uniform Co-operative Field Pea Test organized in that year by J. G. C. Fraser.

The outstanding performance of this line was recognized in the 1956 Co-operative Test and small increases of 3884 A, Arthur and Chancellor were made in 1957 at the Lethbridge, Alberta, Indian Head, Saskatchewan, and Ottawa Experimental Farms and the Special Crops Substation at Portage la Prairie, Manitoba. The seed from the above tests was examined for soup-making qualities by two commercial firms. The reports indicated that 3884 A was superior in quality to Arthur but slightly inferior to Chancellor.

In 1958, two bushels of 3884 A were obtained from W. Hay of the Lethbridge Experimental Farm. One bushel of this seed was increased in 1959 at Portage la Prairie and the second bushel was increased in the Ottawa Valley. Approximately 40 bushels of 3884 A were produced at the two locations. On the strength of its suitability for combine harvesting and high yield of good quality seed, as shown from the results of the Co-operative Field Pea Tests (1) over a period of 5 years, an application for licence was submitted. Licence No. 780 was granted under the name of "Creamette" on February 12, 1960. Approximately 700 bushels of this variety will be seeded in 1961.

AGRONOMIC CHARACTERISTICS

Plant – Average 3½ feet in height, exhibits vigorous growth

Habit of Growth – Indeterminate, flowers borne axially

Flowers – Medium size, white, mostly in pairs

Pods – Medium size, about 2¼ inches long, often slightly curved

Seed – Medium size, yellow, smooth, at times slightly flattened

Maturity – Early to mid-season, earlier than Arthur, approximately equal to Chancellor

Quality – Produces a puree of good colour; cookability superior to Arthur, slightly inferior to Chancellor

Yield – Averaging about 20 per cent higher than Chancellor or Arthur.

[1]Genetics and Plant Breeding Research Institute Contribution No. 59, and Plant Research Institute Contribution No. 133, Research Branch, Canada Department of Agriculture, Ottawa, Canada.

DISEASE RESISTANCE

Creamette was examined for its resistance to the four races of *Ascochyta pisi* Lib., the organism which causes leaf and pod spot of peas. The races (I-IV) and the method of inoculation have been described earlier (2). The results of tests with 40 lines of Creamette seedlings show that, in general, Creamette is moderately resistant to races I and III, slightly susceptible to race II, and susceptible to race IV. Certain lines are resistant to races II and IV.

When all factors are considered, Creamette is more resistant to *Ascochyta pisi* than either Chancellor or Arthur since these two varieties are highly susceptible to races III and IV respectively.

A composite of the above 40 lines is now used for the production of "Breeder's Seed". Some lines are more resistant to *Ascochyta pisi* than others. These are being tested further for yield and disease reaction. Single disease-resistant lines or a composite of such lines may replace the original seed stock if they equal it in yield and quality.

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May 15, 1961

NOTE ON KEYSTONE, A LOOSE SMUT RESISTANT FEED BARLEY

Keystone, a new variety of feed barley was developed by the Barley Project Group for the eastern Prairies from a cross made at the Experimental Farm, Brandon, Manitoba. It is the first variety to be released in Canada with high resistance to the prevailing races of both loose smut and stem rust. Other attributes include high yield, strong straw and non-shattering type heads.

SOURCE OF MATERIAL AND BREEDING METHODS

Keystone was selected from the cross (Vantage x Jet) x Vantmore[3]. Jet was used as the loose smut resistant parent since earlier work had shown this black, two-rowed hulless variety to be the only one completely resistant to all available North American collections of *Ustilago nuda* (Jens.) Rostr. Vantage was chosen as a second parent because of desirable agronomic features and stem rust resistance. In the later crosses Vantmore was substituted for Vantage as it had shown greater resistance to root rot and a number of the foliage-attacking fungi.

Intensive artificial inoculation with loose smut by the pressure-jet method was followed throughout the entire breeding program.

The initial cross, Vantage x Jet was made in 1949. In 1952, an F_5 family was recovered which was homozygous for both loose smut resistance and the six-rowed character, and segregating for stem rust resistance, smoothness of awn, kernel colour and hull adherence. Selected plants from this family were back-crossed twice to Vantmore and, from the resulting progeny, BR. 5502-11 (later Keystone) was isolated in 1956.

BR. 5502-11 was placed in preliminary yield trials at Brandon in 1957 and advanced to the Western Cooperative Barley Test and to regional trials in 1958. On the basis of data obtained a licence was granted in March, 1961.

VARIETY DESCRIPTION

Spike – Six-rowed; mid-long; medium lax; emerges 0-2 inches; erect; lemma awn long, semi-smooth; glume awn equal to length of glume; glume hairs long, numerous; rachis edges with medium number of long hairs.

Grain – Kernels mid-size; hull smooth; aleurone yellow; rachilla long, with numerous long hairs; lateral veins with medium to numerous barbs; basal markings vary from incomplete horseshoe to horseshoe.

Yield – Approximately 5 per cent higher than Parkland and 2 per cent higher than Husky in 50 tests conducted on the Black Soil zone of Manitoba and eastern Saskatchewan.

Height – Equal to Husky; 2 inches shorter than Parkland.

Straw Strength – More resistant to lodging than either Husky or Parkland.

Shattering and Head Break – Less prone than Husky or Parkland.

Maturity – One day later than Parkland; three days earlier than Husky.

Disease Resistance – Highly resistant to loose smut; resistant to covered smut, stem rust, and bacterial streak; moderately resistant to spot blotch; and susceptible to false loose smut, net blotch, and Septoria leaf blotch. Data from one Station in 1959 indicate that Keystone is resistant to scald but susceptible to mildew and leaf rust.

Quality – Eligible for the feed grades only.

ADAPTATION

Keystone appears well adapted to the black soil zones of the eastern Prairies.

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August 10, 1961

NOTE ON APPARATUS FOR MAINTAINING CONSTANT SOIL TEMPERATURES AND SOIL MOISTURES, USED IN STUDIES ON SOIL INHABITING INSECTS AND DISEASES[1]

During investigations on rearing various species of soil insects, equipment was required for controlling both soil temperatures and soil moistures. The apparatus described herein has been used successfully for rearing different species of root maggots, their predators and parasites, and for special studies on plant diseases. The equipment employs the basic unit of a Wisconsin tank as described by Jones *et al.* (3). Since the original apparatus included electrical equipment that is not available today, the present paper describes modern equipment required for controlling soil temperatures in tanks of a given volume.

TEMPERATURE CONTROL

The apparatus as set up for operation is shown in Figure 1. The tanks are 40 inches x 22 inches and 28 inches deep. The sides and bottom consist of a water-tight, inner casing of 20-gauge galvanized iron, painted to resist corrosion, a half-inch of fibre glass insulation, and an outer supporting box made of 1-inch pine. Each tank holds approximately 90 gallons of water.

The electrical equipment required for cooling, including a sketch of the wiring and piping system, is described in the caption of Figure 2. The heating apparatus used in tanks requiring temperatures higher than that of the chamber or greenhouse room is situated in the centre of a tank (Figure 1). This equipment consists of a water immersed thermostat, a pilot-light, and two 250-watt heating elements situated near the bottom of an upright, water-tight, galvanized iron cylinder, 4 inches in diameter and 24 inches long. In the tanks operated at Charlottetown, temperatures were maintained constant to within ±2°F. throughout the tanks by convection and without the use of a water circulating pump.

The cooling and heating systems outlined above were more than adequate for maintaining required temperatures in tanks holding 90 gallons of water. With approximately 35 feet of half-inch copper tubing per tank, a ½-horsepower water-cooled compressor maintained the water temperature in two tanks at 40°F. when the room temperature varied between 80° and 90°F. The heating elements maintained temperatures up to 20°F. above that of the room or chamber.

In shallow temperature baths, Cooper *et al.* (2) reported that a 1½-horsepower compressor with 150 feet of ⅜-inch tubing was required to provide a temperature of 41°F., presumably at room temperatures of 65° to 80°F. However, in the larger tanks used at Charlottetown, a ½-horsepower unit with 35 feet of half-inch copper tubing was more than adequate to maintain a temperature of 40°F. in a room with an air temperature of 80° to 90°F. This indicates that in using the larger tanks there is considerable saving in installation equipment. It is stressed, however, that a

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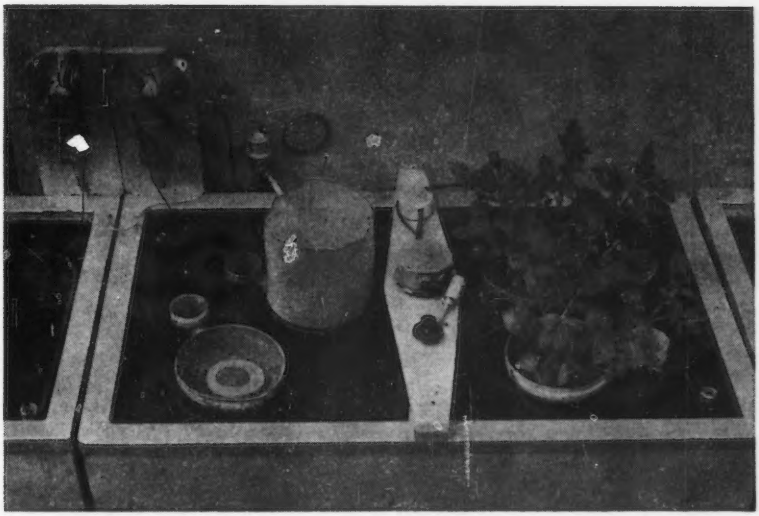

FIGURE 1. Temperature control water tank with 4 moisture control soil cylinders, the 2 on the left being used for insect rearing studies and the 2 on the right for studies on blackleg of potatoes.

water-cooled compressor and not an air-cooled condensing unit should be used; a ½-horsepower air-cooled compressor did not maintain the lower temperatures when connected to two 90-gallon Wisconsin tanks. A series of eight tanks have been in operation at Charlottetown for 3 years without requiring repair.

MOISTURE CONTROL

Moisture levels were obtained by capillary action in soil cylinders, 28 inches long and 7 inches in diameter. Details of the construction and operation of the cylinders have been reported in an earlier paper by Read (4). For tests at one temperature level, up to six cylinders may be placed in one tank. Using a light sandy loam soil and maintaining water table levels at 8 to 22 inches below the surface of the soil in the cylinders, soil moistures at a depth of 2 to 4 inches ranged from near field capacity to near wilting. If plants such as potatoes were being grown in the cylinders, the moisture content of the soil was checked frequently. As the plants developed and transpiration increased, the water table was raised gradually to compensate for rapid loss of moisture. A Bouyoucos Bridge (1), with plaster-of-paris blocks placed about 2½ inches deep in the soil, proved helpful for checking the rate of change of the moisture content of the soil.

The temperature of the soil in cylinders placed in Wisconsin tanks remained more constant than the temperature of the water in the tanks. While the refrigeration or heating mechanisms maintained the water temperature within limits of about 2°F., the temperature of soil inside the

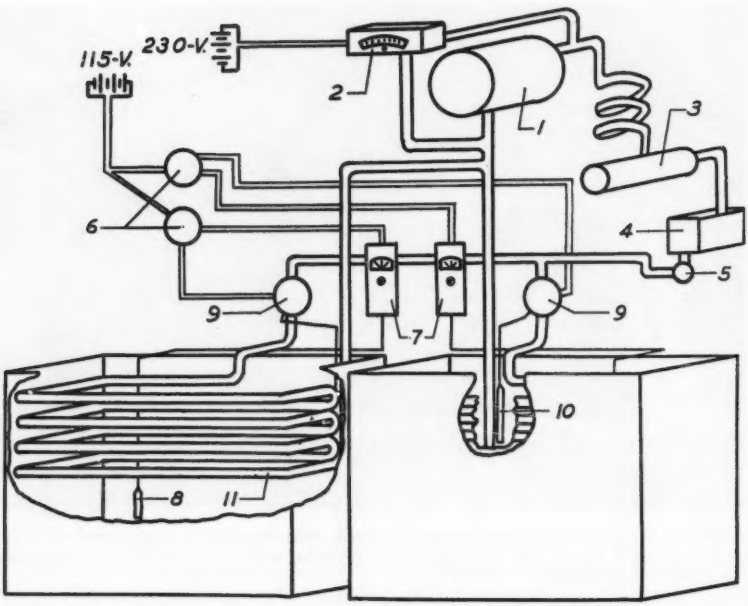

FIGURE 2. Diagram of electrical equipment and copper tubing arranged to control refrigeration in two tanks with one ½-h.p. condensing unit. 1. Compressor. 2. Dual control. 3. Liquid receiver. 4. Dryer (1, 2, 3, and 4 are comprised in a ½-h.p. water-cooled Tecumseh condensing unit, or its equivalent. This unit should be installed in a basement or an unheated room apart from the chamber containing the tanks. 5. Sight glass. 6. Solenoid valve (Automatic Products, Model 73, or equivalent). 7. Variable (40° to 75°F.) temperature control (Minneapolis-Honeywell, model L6021A, or equivalent). 8. Bulb for temperature control, placed 6 inches deep in water and 6 inches from cooling copper tubing coils. 9. Thermostatic expansion value (Detroit valve Model 57300 or equivalent). 10. Bulb for thermostatic expansion valve, fastened to return lead of copper tubing below water level. 11. Thirty-five feet of ½-inch copper tubing coiled around tank with coils 2 inches apart and the upper coil 1 inch below the surface of the water in the tank.

cylinders varied less than 1°F., being about 1°F. above the temperature of the water if refrigeration was being used and the same degree below if the heating system was in operation.

When soil cylinders were set up and desired levels of soil moisture were established, the cylinders were used repeatedly for periods of over a year without variation in soil moisture. However, if the surface soil was removed periodically during tests with insects or diseases, it was found that new soil should have approximately the desired moisture content before being placed in the cylinders; if air-dried soil was used a period of about 2 weeks was required to allow the moisture content to reach a constant level. It was also found that small amounts of water could be sprayed on the soil periodically to simulate field conditions without affecting the soil moisture content at the 2- to 4-inch depth. This reduced evaporation of capillary water in the soil and helped prevent accumulation of salt at the soil surface.

The equipment described herein has been used successfully at Charlottetown in studies on the influence of soil moistures and temperatures on development of *Hylemya brassicae* (Bouché), *H. liturata* (Meig.), the root maggot predators and parasites *Coenosia tigrina* (F.), *Aleochara bilineata* (Gyll.), and *Trybliographa rapae* (Westw.), and on blackleg disease of potatoes and clubroot in crucifers.

ACKNOWLEDGEMENTS

The authors wish to thank J. N. Richard, Technician, Charlottetown, for providing the photographs. Grateful acknowledgement is also made to J. G. Dickson, for information on Wisconsin tanks that have been used so successfully at the University of Wisconsin for more than 20 years.

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NOTE ON CHIGNECTO SWEDE[1]

Chignecto swede was developed by the Forage Crops Section of the Experimental Farm, Nappan. It is somewhat superior to Wilhelmsburger in resistance to clubroot (*Plasmodiophora brassica* Woron). In addition, it has the more desirable purple top, smooth skin and yellow flesh typical of the Laurentian variety table "turnip". This combination should be of value to many potential growers in the clubroot disease areas of the Atlantic Provinces.

ORIGIN

Chignecto is a multiple hybrid for which the first cross between Laurentian and a selected disease-free first generation selfed plant of Danish Giant was made in 1942. Selected plants of the S 3 generation of this cross were backcrossed twice with selected plants of an S3 hybrid of the varieties Wilhelmsburger and Danish Giant. All segregating generations were grown in a long-term disease garden where disease symptom ratings were made. Disease-resistant plants were further selected on the basis of agronomic appearance, cooking quality and seed production. All hybrid and self-pollinated seed was produced in the greenhouse. Yield trials were conducted on disease-free soil at Nappan during the period 1955-59 inclusive. Observation plantings were made at a number of locations under a variety of disease conditions throughout the Atlantic Provinces.

DESCRIPTION

Plant Type – Plant characteristics are similar to those of the Laurentian variety. The root is medium size, globe shape with purple top, smooth skin and pale yellow flesh.

Disease Reaction – Inoculation studies conducted by G. W. Ayers, Experimental Farm, Charlottetown, Prince Edward Island, indicate that Chignecto possesses similar clubroot disease reaction to Wilhelmsburger. It is fairly susceptible to race 1 and immune from races 2 and 3. In disease areas, this resistance is usually expressed at a slightly higher level than it is in Wilhelmsburger.

Yield – The yield of roots suitable for marketing as table turnips has averaged approximately 15 per cent greater than from Laurentian and 5 per cent greater than from Wilhelmsburger on disease-free soil. Where clubroot disease was prevalent only Chignecto produced satisfactory roots.

Table Quality – In several tests conducted at Nappan, Nova Scotia, the average score for all taste factors was slightly lower for Chignecto than for Laurentian. This difference may not be of much importance in other than critical comparisons.

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July 4, 1961

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